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CONTENTS

NUMBERS 1 AND 2, JANUARY-FEBRUARY-MARCH-APRIL, 1938

Purpose of the Food Technology Conference. Samuel C. Prescott.....	1
Recent Developments in Canning Fruit Juices. S. Henry Ayers.....	5
Advancement in Sterilization Methods for Canned Foods. C. O. Ball.....	13
Action of Enzymes at Low Temperatures. A. K. Balls and Hans Lineweaver.....	57
Modern Practice in Fish Preservation by Cold. Louis Berube.....	69
Color of Meat. J. Brooks.....	75
Importance of the Unit Operation Concept in Food Engineering. L. V. Burton	79
Recent Developments in Canning Technology with Reference to Spoilage Control. E. J. Cameron.....	91
Present Use and Future Prospects of Ozone in Food Storage. Arthur W. Ewell	101
Carotene and Ascorbic Acid Content of Fresh Market and Commercially Frozen Fruits and Vegetables. G. A. Fitzgerald and C. R. Fellers.....	109
Spectroscopy in Food Research. George R. Harrison.....	121
Air-Conditioning for Food Plants. James Holt.....	127
Vitamin C Content of Vegetables. VIII. Frozen Peas. R. R. Jenkins, D. K. Tressler, and G. A. Fitzgerald.....	133
Application of Scientific Control in the Bottling Industry. Max Levine.....	141
Gas Storage of Meat and Eggs. T. Moran.....	149
Calorimetric Investigation of Foodstuffs. A. Perlick.....	155
Cristallisation et Dessiccation de Certains Proteides sous l'Action du Froid. Maurice Piettre	161
Essais de "Quick Freezing" Applique aux Grosses Pieces de Viande. Maurice Piettre	167
Contribution to the Theory of Cold Injury to Fruit. R. Plank.....	175
Microbiology in Relation to Food Preservation. Samuel C. Prescott and Fred W. Tanner	189
Determination of Optimum Conditions for Domestic Refrigeration of Foods. Bernard E. Proctor and David G. Greenlie.....	199
Freezing and Cold Storage of Herring. George A. Reay.....	205
Some Recent Advances in Dairy Technology. James A. Tobey.....	211
Apparatus for Measurements of Chewing Resistance or Tenderness of Food-stuffs. N. N. Volodkevich.....	221
Disposal of Food-Plant Wastes. L. F. Warrick.....	227
Utilization of Whey in Foods. B. H. Webb.....	233

ABSTRACTS

Packaging Materials and Their Application to the Food Industry. Allen Abrams	239
Food Preservation in Modern Glass Containers. H. A. Barnby.....	240
Present Trends in Canning. A. W. Bitting.....	242
Current Technological Problems in the Dairy and Ice Cream Industries. A. C. Fay.....	245
Development of a Fiber Container for Fluid Milk. F. F. Fitzgerald.....	248
Research Problems of the Can Manufacturer. William H. Harrison.....	253
Engineering of Pasteurization. C. A. Holmquist and W. D. Tiedeman.....	257
Problems Yet to Be Solved in the Dairy Industry. H. F. Judkins.....	259

Technological Exploration of the Art of Buttermaking. M. E. Parker.....	261
Canning of Cheddar Cheese. L. A. Rogers.....	267
Recent Advances in Brewing Technology. George B. Sippel.....	269
Special Problems in Fish Canning. M. P. Vucassovich.....	273
Better Meals for Tomorrow. Lewis W. Waters.....	276
Research in Metallurgy and Its Significance in Canning. Robert S. Williams..	278

NUMBER 3, MAY-JUNE, 1938

Stability of Vitamin D in Irradiated Evaporated Milk. C. H. Krieger and H. T. Scott.....	283
A Study of Comparative Methods and Media Used in Microbiological Exam- ination of Creamery Butter. G. W. Shadwick, Jr.....	287
Types of Bacteria Surviving in Frozen-Pack Vegetables. A. G. Lochhead and A. H. Jones.....	299
Changes in Iron Content of Musts and Wines During Vinification. E. M. Mrak and J. F. Fessler.....	307
Vitamin C Content of Vegetables. IX. Influence of Method of Cooking on Vitamin C Content of Cabbage. Marylizabeth Wellington and Donald K. Tressler	311
Lactic Acid-Producing Bacteria in Fermentations and Food Spoilage. Carl S. Pederson	317
Heat-Resistance Studies on Spores of Putrefactive Anaerobes in Relation to Determination of Safe Processes for Canned Foods. C. T. Townsend, J. R. Esty, and F. C. Baselt.....	323
Salmonella Food Poisoning—Infection or Intoxication? G. M. Dack and Ellen Davison	347
A Study of Certain Reducing Substances in Citrus Fruits. James B. DeWitt and Barnett Sure.....	351
Iodine Content of Some Ohio Vegetables. Carl Dietz.....	359

NUMBER 4, JULY-AUGUST, 1938

Stability of Vitamin B ₁ of Vacuum-Dried Animal Tissues During Storage. Aaron Arnold and C. A. Elvehjem.....	367
The Antirachitic Effect of Some Foods. E. F. Kohman, W. H. Eddy, Mary E. White, and N. H. Sanborn.....	373
A Note on the Bioassay Technique for Determining Available Iron in Foods. Philip L. Harris and George L. Poland.....	383
Bactericidal Activity of Crotonaldehyde. R. L. Ingersoll, R. E. Vollrath, Ber- nard Scott, and C. C. Lindegren.....	389
Acid-Base Balance of Cereals and Some Related Food Materials. Jehiel Davidson and J. A. LeClerc.....	393
Losses of Vitamin C During Boiling and Steaming of Carrots. Faith Fenton, D. K. Tressler, S. C. Camp, and C. G. King.....	403
Losses of Vitamin C During Commercial Freezing, Defrosting, and Cooking of Frosted Peas. Faith Fenton and D. K. Tressler.....	409
Isolation of Halophilic Bacteria from Soil, Water, and Dung. L. S. Stuart....	417
Detecting Thermophilic Contamination in Skim-Milk Powder. C. M. Sorenson	421
Color in California Wines. I. Methods for Measurement of Color. A. J. Wink- lér and M. A. Amerine.....	429

CONTENTS

Color in California Wines. II. Preliminary Comparisons of Certain Factors Influencing Color. A. J. Winkler and M. A. Amerine.....	439
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NUMBER 5, SEPTEMBER-OCTOBER, 1938

Sources of Lead in Maple Syrup and a Method for Its Removal. C. O. Willits and C. J. Tressler, Jr.....	449
Further Observations on Production of Alcohol by <i>Saccharomyces ellipsoideus</i> in Syruped Fermentations. Leonora Hohl.....	453
Preservation of Grape Juice. V. Pasteurization of Grape and Apple Juices for Storage or Immediate Fermentation. E. Arthur Beavens, Harry E. Goresline, and Carl S. Pederson.....	467
Pectic Enzymes. II. Pectic Enzymes of Tomatoes. Z. I. Kertesz.....	481
•Vitamin Values of Garden-Type Peas Preserved by Frozen-Pack Method. I. Ascorbic Acid (Vitamin C). E. N. Todhunter and B. L. Sparling.....	489
Improved Procedures in Determination of Aldehydes in Distilled Alcoholic Liquors with Schiff's Reagent. Walter C. Tobie.....	499
Variations in Sampling Beef and Pork Roasts for Press-Fluid Investigations. Alice M. Child and Elsie Zohner Moyer.....	505
Adhesion of Potato-Tuber Cells as Influenced by Temperature. Catherine J. Personius and Paul F. Sharp.....	513
Permeability of Potato-Tuber Tissue as Influenced by Heat. Catherine J. Personius and Paul F. Sharp.....	525
Degree of Pigmentation and Its Probable Relationship to the Diastatic Activity of Honey. H. A. Schuette and Janet F. Pearlstein.....	539
Mineral Constituents of Honey. III. Sulphur and Chlorine. H. A. Schuette and Ralph E. Triller.....	543
Nutritive Value for Growth of Some Proteins of Fishery Products. William B. Lanham, Jr., and James M. Lemon.....	549

NUMBER 6, NOVEMBER-DECEMBER, 1938

Action of Gum Guaiacum upon the Animal Organism. Victor Johnson, Anton J. Carlson, Nathaniel Kleitman, and Paul Bergstrom.....	555
Utilization of Calcium of Spinach and Kale. M. L. Fincke and E. A. Garrison	575
Development of Pink Color in Sauerkraut. Carl S. Pederson and C. D. Kelly..	583
A Study of Rate of Decomposition of Haddock Muscle at Various Temperatures as Indicated by Ammonia Content. G. Chapman Crooks and W. S. Ritchie	589
Storage of Shell Eggs. T. L. Swenson.....	599
Inhibitory Properties of Horse Radish Vapors. Milton J. Foter and Ann M. Golick	609
A Note on Yeast Obtained from Slimy Sausage. E. M. Mrak and Lee Bonar..	615
Effect of Coagulation on Press Fluid, Shear Force, Muscle-Cell Diameter, and Composition of Beef Muscle. Mary J. Satorius and Alice M. Child.....	619
Problems in Meat Research. I. Four Comparable Cuts from One Animal. II. Reliability of Judges' Scores. Mary J. Satorius and Alice M. Child..	627
Preparation of Ice Cream Mixes for Home Consumption. W. J. Corbett and P. H. Tracy.....	637

Thermal Processing of Canned Foods in Tin Containers. I. Variation of Heating Rate with Can Size for Products Heating by Convection. O. T. Schultz and F. C. W. Olson.....	647
Chemical and Bacteriological Studies on Ice Cream. Cecily Ruth Grumbine and Evelyn G. Halliday.....	653

PURPOSE OF THE FOOD TECHNOLOGY CONFERENCE

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(Received for publication, November 12, 1937)

For more than 40 years the Massachusetts Institute of Technology, which has sponsored this conference and in whose lecture rooms it is held, has maintained a constant interest in foods and their study. It is a great pleasure, therefore, to welcome you to the first Food Technology Conference ever held in the United States.

Nearly 50 years ago a remarkable woman, Mrs. Ellen R. Richards wife of Prof. Robert H. Richards, who was for nearly a half century the head of our Department of Mining Engineering and Metallurgy, began her lifelong researches into the chemistry of foods and the economics of home management. These pioneer researches established, respectively, a new branch of chemistry in this country and a new profession for women. From this early work in food chemistry and, later, our extended work in the bacteriological aspects of food preservation there has grown here a continuously broadening and healthy interest leading to numerous courses of instruction and to research in what we call Food Technology but which might perhaps with equal correctness be named Food Engineering.

Few people realize how great has been the development in food manufacturing in 40 or 50 years. Those of us whose interests are historical and whose memories can span this period are, in part at least, familiar with the great changes which have taken place and which are still going forward with an impetus that promises much for the future. Whatever may have been the case with some of the aspects of life in the "good old days" it is certainly evident that there has been no period in history when quantity, quality, and variety of good food was so assured as in our own present era.

Within our lifetimes we have seen food supply changes of vast proportions. We have seen the preparation of food commodities grow in numerous instances from a purely domestic or family industry to one of great industrial significance and variety in much the same manner that in an earlier generation our forbears saw the industries of spinning, weaving, leather making, and even the development of machinery grow gradually from a personal skill of handicraft to organized factory production. Until rather recently this change in food supply and food technology has progressed sporadically and

has been the outcome, obviously, of occasional important discoveries and of new and sudden demands, not the least of which has been the need for food for armies and for the rationing of troops in case of war. Who can, for example, measure the impetus given to the canning industry in our sectional strife in 1861-65?

It would be intriguing to recall and to speculate on the theories first propounded by Benjamin Franklin and later developed more fully by Malthus 140 years ago and by Crookes nearly a century later concerning the relation between food supply and necessary limitation of population. Such speculations can lead only to one conclusion, namely, that we can never tell when some new discovery in science will change the whole picture and render too assured prophesy or sweeping prediction of disaster one of the dangerous or at least foolish occupations of man.

The purpose of this conference, however, is not to call attention to the defects, failures, or narrowness of viewpoint of the past. It is, on the contrary, to stimulate a forward-looking attitude and to take account of present stock in trade in the form of proved knowledge and tested experience.

In issuing the invitations for this meeting we have had in mind four objectives:

1. To bring together those interested in the really scientific advancement of food industries whether as teachers, investigators, or manufacturers.
2. To emphasize the essential fundamental unity of character of problems of the industry, since they are all concerned with the scientific control of materials and of factors, chemical, biological, physical, or economic, which affect the usability of foods by mankind.
3. To present some examples of advancement in food technology along specific lines.
4. To emphasize the importance of research and the continuous seeking for new facts and new principles capable of application in the industry.

In a meeting of this brief character only a few of the now many subdivisions of food technology can be considered. We have selected three of these for our discussions.

First comes the advancement in dairy manufactures. In this branch of the food industry we have an excellent example of a great business which in hardly more than a generation has grown from a domestic or farm-unit art, frequently haphazard in character, to a scientifically controlled industry centered in great plans, often pro-

vided with the equipments and accessories of the research laboratory, and controlled with ideals of sanitation, excellence of quality, and high food value.

In the second and third places we shall consider the applications of temperature control in food preservation for here we have two distinct lines of development. These are exemplified on the one hand by the process of canning, in which relatively high temperatures are employed with precision and effectiveness in overcoming microbial infections and the dangers of spoilage, and on the other by the utilization of low temperatures as in refrigeration and quick freezing with the result that the processes of deterioration are suppressed or inhibited.

The recent advances in all these fields are so significant and striking that we may look forward with anticipation and almost with assured satisfaction to the triumphs which lie just ahead of us in these fields of applied research. Chemistry, physics, biology, engineering, and economics are the five points in the star of progress, and with the fullest coöperation and joint effort among them, continued advances in food technology will be assured.

RECENT DEVELOPMENTS IN CANNING FRUIT JUICES¹

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(Received for publication, November 12, 1937)

Developments during the last few years have produced a quality of canned fruit-juice products which the consumer accepts, to a considerable extent, in place of fresh juice.

Consumer demand has grown remarkably during the last five years, probably largely through educational advertising based on the vitamin content, alkalizing properties, and mineral content of fruit juices but also, in part, through the increasing appreciation of the medical profession for the value of canned fruit juices of good quality. This consumer demand could not have been maintained, however, had not developments in packing methods produced canned juices which satisfied the consumer.

In discussing the recent developments in packing fruit juices, attention will be confined largely to the citrus juices, grapefruit and orange juice, with which we have had the most experience.

Not many years ago canned juices were used mainly in mixed drinks. Now they are largely consumed as beverages and replace, to some extent, fresh juices at the home table as well as in hotels and restaurants. This change has taken place, first, because of improved methods of packing which created products with more natural flavors, and second, because of increasing recognition of the value of citrus juices in the diet. Of these two causes, perhaps the most important is the improvement in flavor and keeping quality. As progress is made in this direction, the second cause—recognition of the value of these juices in the diet—will become increasingly significant in enlarging the market.

BEST PRODUCTS FROM FRESH, RIPE FRUIT

Juice from the grapefruit or orange should come only from freshly picked, tree-ripened fruit, free from any unusual defects except possibly size. It is only under these conditions that the full, aromatic flavor is obtained with its natural, balanced sweetness. No "drops" or frozen fruit should be used and the fruit should not be held over 24 hours after picking. Artificially dye-colored fruit is not as good as the uncolored for fruit juice.

¹ Published in full in *Fruit Products Journal* 17, (1937) 41-44. See also *The Canner*, Sept. 25, 1937.

When producing fruit juices it must be remembered that a fine canned product cannot be produced from second- or third-class fruit. At best, any process cannot improve the original quality of the fresh juice. Many times, however, it may at first mask a poor or off-flavor which only shows up more after storage. To secure uniformity in citrus juices it is necessary to watch carefully the acidity and flavor, which will vary with fruit of different sizes or fruit coming from different sections where the soil varies. Some degree of uniformity can be obtained by blending the juice, although it will vary somewhat as the season progresses and the fruit ripens. Overripe fruit must be rejected since juice from such fruit imparts a particularly bad flavor that grows worse upon storage.

In the preparation of a suitable citrus juice for canning, emphasis must be placed on the importance of proper extraction methods. It is desirable to keep the "rag" and as much cell tissue as possible out of the juice. This material adds ingredients which considerably affect the keeping quality of canned juices.

Generally speaking, the extraction method most extensively used has been to split the fruit mechanically and hand spindle the half sections over revolving burrs. Some machines have been developed, with more or less success, to do this mechanically. Attempts have also been made to crush the whole fruit, but these methods have been discontinued, because the oil introduced imparts a strong objectionable flavor which becomes decidedly worse upon storage of the juice.

EXTRACTION BY MECHANICAL METHOD

The method of mechanical juice extraction described here has been developed in connection with a quick-heating process known as the Stero-Vac process, one of the latest and most effective ones used for packing fruit juices. It has been used by the Sun-Dine Company, Inc., for about five years; the following descriptions cover their methods.

There are three steps in the citrus-juice extraction: grating the fruit to remove the oil, pressing the whole fruit, and straining the juice to remove seeds and pulp.

The grater consists of two horizontal revolving discs about four feet in diameter, each of which is covered with stainless steel fillet. Suspended above each disc a distance of about one quarter of an inch is a spiral, consisting of stainless steel fillet properly supported so as to be rigid. The horizontal discs supported by bearings revolve while the spiral suspended above them is stationary. In operation the horizontal discs rotate at a speed of about 100 revolutions per minute and revolve in opposite directions. The fruit rolls into the center of the first disc and is thrown by centrifugal action of the revolving disc against

the fillet of the stationary spiral and so is turned over and rolled from the center of the first revolving disc to the outside.

As the fruit travels through the spiral, its surface is punctured and grated by the protruding perforation of the fillet and the oil is released from the skin of the fruit. As the fruit leaves the outside of the first revolving disc it is transferred to the outside of the second revolving disc and the grating action is continued as it passes through the spiral from the outside to the center of the disc. At the center it drops through a hollow vertical bearing and rolls to a continuous press. During the passage of the fruit through the grater, a fine spray of water is applied to it on the second disc to wash off the grated peel and oil.

The continuous press consists of two heavy discs covered with stainless steel. These discs, about three feet in diameter, revolve in the same direction and at the same speed and are so shaped that they come closely together for a short distance on one side. Whole fruit rolls from the grater into one side of the press, is crushed, and the juice is extracted. After being pressed, the crushed peel is lifted off the disc by a plow to prevent absorption of juice, and the juice flows into a stainless steel trough which surrounds the lower disc.

The juice then flows to a finisher, a mechanical strainer consisting of stainless steel screen with fine holes. The pulp and seeds, which are strained out, are removed by the action of paddles which carry them to an outlet pipe. The purpose is to remove the seeds and pulp effectively without losing too much juice.

The next step is deaeration. Oxygen is deleterious to the keeping quality of fruit juices and its removal is of vital importance. The amount in fruit juices varies with the method of extraction, and reamed juice contains considerably more than pressed juice. It not only should be removed from fruit juices, but removal should be complete and as quick as possible. Subsequent to its removal, the juice must be so handled that it is kept out.

As the juice leaves the finisher it flows continuously to a stainless steel tank near the deaerating unit which plays a very important part in packing fruit juices and serves a dual purpose in the Stero-Vac process. The deaerating unit consists of a steam chest connected to a separating chamber which, in turn, is connected to a condenser. All this equipment is constructed of stainless steel. The steam chest consists of a large tube enclosing about 20 straight pipes. Juice flows through these pipes which are surrounded by hot vapor.

Juice is drawn from the receiving tank through the pipes in the steam chest by a high vacuum, about 28 inches, and during its passage it boils under the vacuum. It leaves the steam chest under high

velocity and passes into the separator, also under the same vacuum, where the juice falls to the bottom and the gas and some vapor pass to the condenser. By this process of continuous boiling under a high vacuum the oxygen and part of the carbon dioxide are removed from the citrus juice.

This accomplishes one purpose of the deaerating unit, namely, the removal of oxygen. At the same time, a definite percentage of water is removed. This is to compensate for the water added by condensed steam during the Stero-Vac heating by steam injection, which will be described a little later. The amount of water removed in the deaerating unit is definitely controlled by the temperatures and rate of flow of the juice and is checked by the condensate from the condenser which is collected in calibrated receiving tanks.

The juice is pumped out by a stainless steel vacuum pump to the filler, which is so constructed that the juice from the deaerating unit may be broken to atmospheric pressure in an inert gas, such as nitrogen.

For the packing of grapefruit juice, plain tin cans are used. This is necessary to prevent darkening of the juice and the production of off-flavors. With orange juice it is necessary to use lacquered cans in order to prevent flavor changes. Exposure to tin gives orange juice an off-flavor immediately after processing and this flavor change becomes decidedly worse upon storage. Orange juice is very corrosive to ordinary lacquers; extensive studies are being conducted to find more resistant lacquers. A perfect inside can coating for orange juice has not as yet been found. Certain lacquers give very satisfactory results, however, from a commercial standpoint.

DISCUSSION OF STERO-VAC PROCESS

Since the commercial introduction of the quick-heating Stero-Vac process in about 1931, progress has been made in other flash-pasteurizing systems for the citrus juices. In general, these consist of some form of deaeration followed by a flash-heating of the juice, partial cooling, and filling into previously steamed cans.

The Stero-Vac process is distinctly different from these flash-pasteurizing systems in that the juice is flash-heated in the final container in the practical absence of air. This is accomplished by heating in the can by steam injection through a patented valve. This valve is formed in the end of the can and contains a floating disc which is forced, after the Stero-Vac treatment, against a special sealing compound and crimped into permanent position and tightly locked. In the commercial lines these valve ends are double seamed on the filled

cans in the regular manner. At this point, therefore, the can of juice is completely sealed except at the valve.

The juice leaves the deaerating unit at a temperature of 37.8°C. (100°F.). The temperature is slightly lower in the filled can. After filling and seaming the valve ends on the cans, they are then run through a short exhaust box where the temperature is raised to about 43.3°C. (110°F.). From this heating bath the cans go to the Stero-Vac machine which consists of a turret carrying headers with which the cans are held in contact. Vacuum and steam are applied through these headers and the turret revolves in a vertical plane so that the cans are turned upside down, the vacuum and steam being applied through the valves in the ends of the cans.

The filled cans with the valves open are then fed automatically upon the Stero-Vac machine at the bottom of the turret so that the valve is in contact with the header. During the first part of the Stero-Vac cycle the can receives a high vacuum as the turret revolves. The object of preheating the juice to about 43.3°C. (110°F.) is to cause it practically to flash-boil under the high vacuum the filled cans receive on the Stero-Vac machine. This removes the oxygen from the head space in the can prior to steam injection. This vacuum is applied first when the can is in an upright position and continues until the can is tilted and the liquid content almost reaches the valve. At this point the vacuum is cut off and steam is injected directly into the can through the valve. As the liquid is then in contact with the valve it is violently agitated by the flow of steam into the can and is rapidly heated. The can continues with its steam treatment until it has been turned upside down and carried for about three-quarters of a complete revolution of the turret, when it is automatically lifted off the header. As it is removed from the header, the disc in the valve is suddenly snapped into place against a special sealing compound by the sudden change in pressure from that built up in the can and atmospheric pressure.

The can, at this time, is temporarily sealed as the disc is held in place by the pressure in the can. It is permanently sealed by its subsequent immediate passage through a clinching unit in the front of the machine. This consists of a series of jaws which grip the side of the valve and compress the tin below the valve disc, thus forcing it into a locked position against the sealing compound.

When the Stero-Vac process is applied to fruit juices, the complete cycle on the machine takes about ten seconds, with approximately eight seconds of steam treatment. During this period the temperature of the juice in the can is raised from about 43.3 to 98.9°C. (110 to 210°F.) or an increase of approximately 100°F.

By this method of rapid flash-heating no flavor changes are produced, enzymes are inactivated, and the vitamin C content is unaffected. Corrosion of the tin plate of the container is reduced. The process enables more efficient heat usage and has labor-saving features.

In the canning of fruit juices it is essential that the juice be rapidly heated in such a manner as to produce commercial sterility without flavor change. It is also equally important that the juice be cooled as rapidly as possible.

Following the Stero-Vac flash-heating process in the can, the containers run immediately into a cooling system in which the cans are submerged in water and slowly rotated in a trough to which fresh water is supplied in a coarse spray over its entire length.

As the cans leave the Stero-Vac machine, the temperature of the juice is about 98.9°C.(210°F.) and it is under about 17 pounds of pressure. At the end of the cooling line the temperature is about 32.2 to 35°C.(90 to 95°F.) and the cans show a vacuum of about 22 inches. The cans are then conducted over a runway and rolled at high speed around sharp turns to throw off adhering water and then run through the labelling machine into the final packing cases. The juice is not cooled below 32.2°C.(90°F.), in order that this temperature will assist in causing any moisture on the can to dry rapidly.

It is important that fruit juices be extracted by a continuous process and canned as rapidly as possible. In the Stero-Vac system, as practiced in the Sun-Dine Company's plants, the entire process takes approximately five minutes and is continuous.

The same system may be applied to all fruit juices as well, the extraction method being the only procedure which has to be varied. The author believes that many desirable vegetable juices could be prepared by this method. Tomato juice processed by this flash-heating system in the final container has a fresh uncooked flavor, entirely different from the usual canned tomato juice.

One of the problems in packing pineapple juice is the change in color and the loss of flavor which take place during storage. We have had pineapple juice processed by the Stero-Vac method, packed for five years, which showed no color change and no change in flavor so far as could be noted. It seems, therefore, that some of the old problems encountered in packing juices can be solved by recent developments in packing methods.

In closing, we wish to call attention to the fact that the use of the Stero-Vac process is not confined to fruit juices. Many fruits have been processed by this method with the result that they retain most of their natural flavor and texture.

Under experimental conditions and by the use of temperatures above the usual ranges, evaporated milk, as well as whole-grain corn, peas, string beans, and lima beans have been rendered sterile from a commercial standpoint. Evaporated milk requires a cycle on the Stero-Vac of about 15 seconds and the vegetables mentioned, a cycle of 20 seconds. Naturally, the effect of this short sterilizing period is astonishingly different from the product resulting from the long periods of retort processing. Evaporated milk, for example, retains its original color and flavor while the vegetables far more nearly approach the fresh products, particularly in flavor and texture.

We believe that through quick heating and cooling processes the canning industry has revolutionary possibilities ahead of it.

ADVANCEMENT IN STERILIZATION METHODS FOR CANNED FOODS

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(Received for publication, November 12, 1937)

INTRODUCTION

Webster's Dictionary states, "The sterilizing of a medium implies complete destruction of all germs in it." The Century Dictionary says that to sterilize is to render free from living germs. Dictionary definitions of this term do not apply, however, when foods in general are being considered. After years of endeavor, technologists of the canning industry are still trying to find a concise definition of the term "sterilize" as applied to canned foods. In the absence of such a definition let us quote from Tanner (1932) in regard to the subject of sterilizing processes:

"Some canning technologists have modified the term 'sterilization' to indicate the bacteriological condition attained in some foods by processing. Since the term 'sterilization' implies the absolute destruction of all living organisms, and since this condition may not be attained in some processed foods, the term 'commercial sterilization' has been introduced."

Tanner stated that "commercially sterile" canned food may contain viable spores of a type, such as the thermophilic, which will not develop under conditions that are normally maintained during storage of the food. Some of these spores are so highly resistant that, to destroy them by heat, one might overcook the food to such an extent that it would be unsuitable for sale. It is with the subject of commercial sterility that this paper will deal.

HISTORICAL REVIEW

Before Appert

Heat is the most valuable preserving agent for foods now known. Before heat came into studied use as the result of investigations by Nicolas Appert, humanity availed itself, more or less voluntarily, of food preserved by five other agents, namely, asepsis, fermentation, dehydration, addition of chemical substances, and low temperature. Preservation by these means came often as a result of Nature's processes—entirely without control by man. Asepsis, especially, was almost entirely the result of natural phenomena.

Asepsis implies the absence of microorganisms in a food and a continued protection of the food from contamination with microorganisms. Before the time of Appert, the only preservation of food according to this principle was that accomplished by Nature in covering sterile food substance with a shell or a cellulose skin that cannot be penetrated by bacteria.

None of the other four preserving methods gives a food that is free from bacteria. Food preserved by fermentation, dehydration, low temperature, or addition of chemical substances is merely maintained in such a state that growth of bacteria present within it is prevented. Conditions that are produced in the food by these methods and on which the preservation depends, must be maintained as long as the food is to be protected from bacterial spoilage. To preserve the condition which inhibits bacterial growth, however, it is not necessary to seal the food within a covering that protects it from contact with air or with other objects; for example, fruits preserved by the addition of sugar or meats preserved by the addition of salt will not suffer bacterial decomposition so long as concentrations of the preserving substances are maintained at the necessary high points. Nor will meats or vegetables that have been preserved by dehydration undergo bacterial spoilage so long as the moisture content of the food is kept at the necessary low point. These foods do not have to be protected from bacterial contamination to keep them in a state of preservation.

Before Appert, man was not able voluntarily to preserve food by means of asepsis; nor was he able to avail himself of low-temperature preservation except when low temperature was provided by Nature. All the means that he could use voluntarily gave finished products that were vastly different in appearance and flavor from the original material.

Appert, in enumerating the reasons why a heat-sterilizing process was desirable, said that desiccation takes away the odor, changes the taste of the juices and hardens the fiber; sugar conceals, or in part destroys, other flavors and is very costly; salt communicates an unpleasant acerbity to substances, hardens the fiber, and renders it indigestible; and vinegar can be used for only a few articles.

Appert's Work

Discovery of the principle of sterilization by heat and initial development of the art of heat processing by Appert constitute the most outstanding accomplishment in the history of food preservation.

"Appert (1810) described the four important steps in his method of processing food as follows: "(1) In inclosing in bottles the substances to be preserved; (2) in corking the bottles with the utmost

care, for it is chiefly on the corking that the success of the process depends; (3) in submitting these inclosed substances to the action of boiling water in a water bath (*balneum mariae*), for a greater or less length of time, according to their nature, and in the manner pointed out with respect to each several kinds of substance; (4) in withdrawing the bottles from the water bath at the period prescribed."

What food Appert first preserved is uncertain, as a wide variety of foods was discussed in his book. He learned in his experimental work that it is necessary to make a distinction between acid and non-acid foods in regard to the length of time they should be processed.

Appert's explanation of the efficacy of his process was that "Fire has the peculiar property, not only of changing the combination of the constituent parts of vegetable and animal productions, but also of retarding, for many years at least, if not of destroying, a natural tendency of those same productions to decomposition."

Tin Cans and Autoclaves

Between 1815 and 1820, 20 years after Appert began to work intently to develop a method of preserving food with the use of heat, work in England, which was inspired by his accomplishments, led to the use of tin containers for sterilized foods. Appert also used tin cans later.

Some time before 1830 the autoclave was introduced as equipment for cooking canned foods under pressure. According to Bitting (1916) the first attempt to use the pressure principle in cooking was made by Papin, a Frenchman, in 1681. He used an iron pot called a marmite, which had a cover that could be clamped down to withstand moderate pressure. The use of the autoclave in canning was apparently started by Nicolas Appert; and in 1852, Raymond Chevallier-Appert, successor to Nicolas in managing the business established by the latter, was granted a patent on an autoclave equipped with a pressure gauge. Others credited with the early use of water and steam under pressure for canning are Fastier in 1839, and Isaac Winslow in 1843.

Use of Salt Bath

Owing to difficulties encountered in controlling the operation of the autoclave and the attendant danger of explosion, the autoclave did not grow rapidly in favor. However, during this period of increasing recognition of the economic advantage of processing nonacid food substances at temperatures above 100°C. (212°F.) came the realization of the possibility of using a salt bath, without pressure, for processing at temperatures above the boiling temperature of water.

Bitting (1916) said that the English are credited with first using sodium chloride in water for processing canned food, although there is no available record to substantiate the fact. He stated further that the first French record credits Favre with using salt in France in 1850, and Collin with having used calcium chloride the same year. According to Orem (1914), the introduction of calcium chloride for canners' use in the United States is credited to Isaac Solomon, a canner in Baltimore, about 1860-61. Orem stated, "A temperature of 115.6°C.(240°F.) was easily obtained and in consequence, the time of process was reduced from five to six hours necessary by former methods, to 25 or 40 minutes, dependent upon the kind of food to be cooked, while the output of the cannery was increased from 2,500 to near 20,000 cans per day." Losses owing to explosion of cans during treatment were, however, considerable.

Development of the Autoclave

During these years the technique of controlling the operation of the autoclave was being improved and this type of equipment was soon thereafter brought into commercial use. According to Orem, A. K. Shriver, a canner of Baltimore, in 1874, invented the combination closed-kettle for cooking with superheated water or live steam. This was the first canning retort that received its steam from an outside source.

In regard to retorts, Prescott and Underwood (1896) stated: "The introduction of digesters or 'retorts,' about 1870, was the next and most recent step in the development of sterilizing apparatus. By their use an actual temperature of 250°F.(121°C.) may be easily obtained both inside and outside the can; so explosions are avoided. The corresponding pressure is 14 pounds. The only precaution required is to reduce the temperature and pressure cautiously. They have not been accepted as satisfactory, however, on account of the darkening of the goods caused by long continued heating. It is probable that retorts are now used less than formerly in some quarters, through ignorance of their effectiveness, yet it is well known that many packers are using them with excellent results."

Relation of Sterilizing Process to Quality

Heat processing under pressure remains today the most satisfactory method of sterilizing canned foods of low acidity. The point touched upon by Prescott and Underwood as to the effect of long-continued heating in impairing quality of the goods is important; and this factor has had a major influence on developments in the field of pressure processing. While Nicolas Appert stated that im-

provement of quality of preserved foods was one of the primary incentives which led to the discovery of heat sterilization, it must not be assumed that this ceased to be a problem after his discovery and during development of the new art. Today, without a shadow of a doubt, improvement of quality stands first as a stimulus to further development of the art.

Bacterial Contamination and Commercial Processing

Even through the period when the improvements already noted were being made, and for many years thereafter, the procedure followed in processing was the result of "cut and try" operation. Appert had used a process of an hour at 100°C.(212°F.) with long coming-up and cooling periods because of the glass containers, but by 1860, when higher temperatures were first used, the length of maximum processes at boiling temperature had increased to five or six hours. The processes that were successful when used by Appert on a small scale proved inadequate in commercial use. This was due to the fact that food packed commercially on a large scale had greater bacterial contamination than the foods packed by Appert. Aside from the change in technique resulting from introduction of the tin container, an increase in processing time, brought about because of spoilage, was the only advancement made in the art of processing between the time of its discovery and the time of introduction of the calcium-chloride bath. Prescott and Underwood (1896) said that the advent of tin cans caused the first great stride in the business, as it opened a large field for work in the preservation of meat, vegetables, and fish.

PROCESSING ACID PRODUCTS

In processing fruit and other products containing enough acid to give them a pH value of 4.5 or less, we are still unscientific. The requirement of a process for acid products is usually stated in terms of a temperature that must be attained at the center of the can without regard to the length of time required to attain that temperature; for example, the minimum process is usually described as that which brings the temperature of every particle of the product to 82.2°C. (180°F.). This condition exists because of the facts that microorganisms capable of causing spoilage of acid products have such low heat resistance that they are destroyed during a mild process at or below the boiling point of water, and the length of the sterilizing processes for some fruit at 100°C.(212°F.) or lower can vary considerably without materially affecting the quality of the canned product. Consequently, since it has not appeared necessary to establish processes

that will sterilize acid products with the least effect upon quality, comparatively little study has been devoted to this problem.

Even less is known about processing semi-acid products having pH value within the range of from 4.5 to 5.5 than is known about processing acid products. We regard the accumulation of information along this line as important, and it is probable that this field of study will not be neglected much longer.

PROCESSING NONACID PRODUCTS

So far as the nonacid products (vegetables, sea foods, meats, etc.) are concerned, sterilizing retort processes often impair quality of the product. The industry has long recognized the importance of studying processing of these products in order to learn what sterilizing processes will have the least adverse effect upon quality of the canned product. In fact, almost all our processing studies have been confined to this field. Since the introduction of the pressure retort in 1874, the only important advances in the technique of commercial canned-food processing have been introduction of a preheat treatment of the food before sealing the can; improvements in retort manipulation; introduction of the rotating reel-batch process retort, such as is used with evaporated milk; and introduction of the continuous pressure cooker of the Anderson-Barngrover type, the principal use of which is also with evaporated milk. The preheat treatment, which is facilitated greatly by the use of the sanitary-type can, brought about almost complete elimination of double retorting and broguing, which were formerly necessary in order to produce vacuum in the finished can of food.

An increasing amount of attention is now being given to the so-called "high-short" methods¹ of processing nonacid foods, and the indication is that these methods will in time supplant present ones.

CHANGING IDEAS OF HOW PROCESS OPERATES

Processes for canned food have always been designated in terms of time and temperature. For a simple process, the temperature named is the temperature held uniformly in the heating medium dur-

¹ This paper was prepared with the use of the word "flash" to designate the "high-short" process, and the word "flash" was used when the paper was read in the Food Technology Conference. Several objected to the use of the word because it is a misnomer. The objections were well founded; but the only strictly accurate designation, "high-temperature short-time" is cumbersome. At the conference session on September 15, Dr. J. H. Shrader, formerly of the Sealtest System Laboratories, suggested that the designation "high-short" be used. In the author's opinion this suggestion merits action, and therefore, he is venturing, in the absence of any form of official sanction of the term, to substitute it in this paper in place of the word "flash." This is done for the purpose of discouraging the improper use of the latter term.

ing the part of the process that exerts the major sterilizing effect on the food in the container. The time named designates the period during which the temperature is held; for example, a process of 15 minutes at $115.6^{\circ}\text{C}.$ ($240^{\circ}\text{F}.$) is one in which the medium surrounding the can is held at $115.6^{\circ}\text{C}.$ for 15 minutes.

Appert's description of his processes shows him to have been a scientist. Although his careful specifications of the procedure to be followed show that he appreciated the fact that coming-up time and cooling time are important in a process, it seems that many industrial canners later lost sight of this fact. The factors of coming-up time and cooling time increased in importance when high temperature processing of foods came into vogue; nevertheless, except in rare instances, canners of 35 years ago discounted effects of coming-up time and cooling time upon the process. Moreover, since the process was described merely as a process of x minutes at $y^{\circ}\text{F}.$, the conception generally accepted was that every particle of the food was subjected to a temperature of $y^{\circ}\text{F}.$ for x minutes.

Scientists of the canning industry almost 40 years ago began to realize that to understand processing they must know the rate at which temperature rises at that point in the can last reached by the heat traveling into the can during the process. Thus the line of investigation known as heat-penetration study was born. The earliest scientific reference to this subject I have found in the literature is by Prescott and Underwood (1898). By the use of maximum registering thermometers, they found that at the center of a can of corn being processed in a retort at $118.8^{\circ}\text{C}.$ ($246^{\circ}\text{F}.$) a temperature of $108.3^{\circ}\text{C}.$ ($227^{\circ}\text{F}.$) was reached in 30 minutes when the temperature of the corn at the beginning of the process was between 82 and $88^{\circ}\text{C}.$ (between 180 and $190^{\circ}\text{F}.$). Temperatures of 114 and $118.8^{\circ}\text{C}.$ (237.2 and $246^{\circ}\text{F}.$) were registered at the end of 45 minutes and 55 minutes, respectively. The following year Underwood (1899) said that in a large number of cases of spoilage, especially in its first stages, the souring may be found only at the center of the can. He stated: "The corn may be sweet at the top of the can, but on taking a sample from the center, souring will be found. Should these cans stand at a favorable temperature for some time the infection will become general throughout the whole contents. This proves that the heat sufficient for complete sterilization has not penetrated through the central portion on account of the low conducting power of creamed corn."

HEAT-PENETRATION EQUIPMENT

Apparatus for measuring heat penetration has undergone many changes. Maximum-reading glass thermometers, ordinary mercury in

glass thermometers, other expansion-type thermometers, chemical indicators which permanently change color upon reaching a certain temperature, materials which melt within the range of processing temperature, and thermocouples of various designs have been used. The two major advances in the art were the change from the use of glass thermometers to the use of thermocouples, and improvements in thermocouple equipment and in operating technique with this equipment.

Kochs and Weinhausen (1906) followed the lead of Prescott and Underwood in using maximum registering thermometers; while Biting and Biting (1917) adopted a thermometer with which the temperature could be read continuously during the heating of the can. Magoon and Culpepper (1921-1922) used both the continuously indicating thermometer and the thermocouple.

While Biting and Biting apparently were the first to use the thermocouple in a heat-penetration test, they did not carry on with this instrument because it was thought to be too delicate for practical purposes. Thompson (1919, 1922) used the thermocouple successfully to establish experimental heat-penetration curves to check theoretical calculations about the time the National Canners Association, under the direction of Bigelow (1920), entered upon a heat-penetration program that has been responsible for bringing the thermocouple to a point of high efficiency, and to recognition as the best available instrument for heat-penetration tests.

Bigelow first used thermocouples having the two wire leads of each couple sheathed in a tube made of lead. Later, pressure rubber tubing was substituted for the lead tubing. A special fitting was used to permit the wires of a set of thermocouples to pass through the wall of a retort without causing the retort to leak when under pressure. The thermocouple junction within the can was supported and held in place by brass and copper tubing.

The laboratory of the Glass Container Association used a thermocouple with a bakelite support for the junction within the container. A bakelite support of improved design was introduced by the research laboratory of the American Can Company, which, in 1922, had inaugurated the use of thermocouples with wires sheathed in asbestos. With the asbestos sheathing, it was no longer necessary to use the special fitting for passing the wires into the retort. These improvements brought the thermocouple to its present form.

INFLUENCE OF IDEA OF HEAT PENETRATION

When the idea of knowing the time at which the center of the can reaches the temperature of the medium surrounding the can (known

as the processing temperature) became established in the minds of canners, it seemed at first to foster the thought that a process should be described by designating the time during which the heating is continued after the center of the can reaches a given temperature. The inclination of the canner, when he began to grasp the significance of the heat-penetration idea, was to say that a certain product—corn, for example—must be processed in such a way that the corn at the center of the can is at the processing temperature, or at least at a specified slightly lower temperature, for a certain number of minutes. He based his specifications on the first heat-penetration results to come to his attention. Such a description of a process has proved to be satisfactory for acid products. In fact, although unscientific, it is still used in specifying processes for fruits. Owing to variation in the rate of heat penetration for different products and for cans of different sizes, combined with the necessity of using heavy processes, however, this method of specifying processes is not satisfactory for nonacid products. The error in processes established on this basis increases with the increase in processing temperature and is consequently greater for nonacid foods than for acid foods. A process for large cans of nonacid food thus established on the basis of heat-penetration data for a small can, would have a great excess of sterilizing value and would probably impair the quality of the food very much by overcooking.

BACTERIOLOGY OF PROCESSING

Bacteriological laboratories test bacterial spores to determine their strength in resistance to heat. Thus they provide knowledge of the time necessary to destroy spores at different temperatures. The time-temperature relation for the death points of spores is a continuous function, which fact signifies that there is no temperature within the range of those used in processing canned food that can be regarded as a critical temperature. This shows the fallacy in the idea that one can designate, within the range of lethal temperatures, any one temperature that must be reached by a food in a thermal process if the food is to be made sterile. Any temperature that is lethal to the spoilage organisms under the chemical and physical conditions present in the food will be effective in sterilizing the food, provided the food is subjected to the temperature for a sufficiently long time. This fact means that heat at all lethal temperatures contributes something towards the sterilization of the food; therefore, sterilizing effect begins to be exerted at the center of a can as soon as this point reaches a lethal temperature.

ESTABLISHING COMMERCIAL PROCESSES

Through the use of bacteriological and heat-penetration data, scientific methods have been introduced into the establishment of processes for canned food. Two courses of procedure are followed, namely, the canning experimentally of food that has been inoculated with bacterial spores of known resistance, and mathematical calculation of the sterilizing value of processes.

Russell (1895) gave us the record of the first experimental inoculated pack. He made this in a study of spoilage in peas, and by the results of the pack he was enabled to prove that the spoilage was due to bacteria.

Experimental inoculated packs have been used extensively in establishing the processes for nonacid foods that are recommended by the National Cannery Association (1937). Meyer (1931), Lang (1935), and Cameron (1936) described the use of this type of experiment to translate laboratory test-tube findings and calculations into terms of commercial practice. They also generously appraised the calculation method of determining processes, as developed by Bigelow (1920), and Ball (1923, 1927, 1928).

Process calculations are of great assistance in studying the relative effect of equivalent processes at different temperatures upon quality of a product. These data have contributed largely to the important information that, in proportion to their lethal effect, the higher temperatures have less effect in impairment of the food than the lower temperatures. We have determined experimentally, for example, that heating at 115.6 and 143.3°C. (240 and 290°F.) respectively, produces an equal effect upon color and flavor of evaporated milk when the heating at 143.3°C. has four times as great a calculated sterilizing value as the heating at 115.6°C. For four processes at higher temperatures having equal effect on quality of evaporated milk, the calculated ratios of sterilizing value, referred to the 115.6°C. (240°F.) process, are 126.7°C. (260°F.), 2.2; 132.2°C. (270°F.), 2.8; 137.8°C. (280°F.), 3.7; and 143.3°C. (290°F.), 4.4. These ratios are from the results of extensive experiments conducted during the past six years on high-short sterilization of evaporated milk. This work will be discussed further.

MEASURES TO PRESERVE QUALITY

Fractional Sterilization

In the absence of a means to speed up the rate of heat penetration a modification in processing technique, based upon Pasteur's method of sterilizing bacteriological media, found some favor among canners for a number of years. Pasteur's method, now known as pasteuriza-

tion, consisted of heating the medium for a half hour at a temperature of 57°C. (134.6°F.) or higher. The modified method, by which processing at boiling water temperature, or lower, was applied to the sterilization of nonacid canned foods, was introduced by Professor Tyndall. This was the fractional, or intermittent, method of sterilization. Macphail (1897) credited Tyndall with enunciating the principles of this method in 1878. Expressed briefly, fractional sterilization consists of three or more heat treatments on successive days, each of which consists of a one- or a two-hour cook in boiling water. It was assumed that between heating periods, spores not destroyed by previous heating would develop into the vegetative forms which would be destroyed during the next heating period.

When it worked, as apparently it did sometimes, it was the answer to the canners' prayer for a low-temperature process that would preserve the quality of the product and would not consume an excessive amount of time.

Today, fractional sterilization is entirely discredited for use with nonacid foods. Its unreliability lies in the fact that microörganic spores frequently lie dormant (that is, do not develop into the vegetative form, which is of low heat resistance) for considerable periods of time. The length of the period of dormancy cannot be controlled, nor can it even be known with certainty. In laboratory experiments it has been found to vary from a few hours to as long as three years in food that is held at ordinary room temperatures. There is a possible objection to the method from another standpoint. Since the spores must germinate during the holding periods between boiling treatments, the material must be held at some temperature that is favorable for growth. If the spore development is rapid, partial decomposition of the food may take place during the holding periods.

Scientific Retort Manipulation

For production of retort-processed foods having color and flavor which are most nearly like those of home-cooked foods, retort operation must be such that (1) every container receives the same treatment, (2) the retort is heated rapidly to its holding temperature, and (3) the containers are cooled rapidly.

Since the beginning of the era of retorts, four steps have been taken which stand out for their importance to the advancement of the technique of "still"-retort operation. These are as follows:

1. The introduction of the indicating pressure gauge and the indicating thermometer as retort equipment.
2. The change from generation of steam within the retort by external heating of the retort, to the piping of externally generated steam into the retort.

3. The studies of heat penetration into the container and of temperature distribution within the retort, especially the studies made with thermocouples.
4. The introduction of automatic temperature and pressure controllers.

The thermometer and the pressure gauge were used on the retort for many years before the value of this combination of instruments was appreciated. Nevertheless, their use from the beginning did result in an increase in uniformity of processes and a reduction in danger of bodily injury from explosion of retorts. Later their use was essential to the success of the scientific studies of temperature distribution within retorts, even though the actual temperature measurements in the most successful of these studies were made with the thermocouple. Ball (1924) explained how the results of these studies are applied to bring about uniform treatment of all containers within a retort. Carefully planned distribution of steam supply within the retort and adequate venting to remove the air are the essential factors.

Under certain processing conditions, satisfactory temperature distribution is more easily obtained with water than with steam as the heating medium. An outstanding example of such conditions is found in the processing of glass containers in which the problem of control is complicated by the necessity of superimposing pressure above the vapor pressure of the steam. The advantages of using water in processing glass containers have been pointed out by Parcell (1927, 1929, 1930), Ford (1930), and Parcell and Cruess (1932). Raney (1931) and the author of an anonymous article in *Canning Age* (1927) describe a modified process with features developed by the Anchor Cap and Closure Company. In this process the superimposed pressure is exerted by air entrapped in the retort when it is closed.

When the requirement of superimposed pressure does not present itself, steam is generally the more satisfactory heating medium. It has the advantages over water of lower cost and the possibility of more rapid heating of the retort and more rapid cooling of the containers.

Automatic Control of Processes

The piping of externally generated steam into the retort is essential to the use of automatic controllers for the heat supply. From Amdursky's (1926c) review of the history of retort control, starting with the time when the common form of pressure-indicating gauge was the only instrument used, we list the various instruments now used in the order of their introduction into the field. The first companion of the pressure indicating gauge was the pop safety valve; after this came the indicating thermometer; then the mechanically automatic instruments appeared in the following order: self-operated pressure regulator, air-operated pressure regulator, recording ther-

mometer, and air-operated temperature regulator. Illuminating discussions of the value of these instruments have been written by Amdursky (1926b), Hall (1932), Harting (1927), and Taylor (1926). Other writers, Amdursky (1926a), Brendol (1933), Lyon (1926, 1929), Schmid (1932a), and anonymous writers in *Western Canner and Packer* (1933a, 1933b, 1933c, and 1933d), and *Food Industries* (1936a, and 1936b) discuss particularly the care and use of the instruments.

The value of a mechanical controller is measureable directly in terms of the amount of manipulative activity it can take out of the hands of a human operator. The intrinsic worth of relieving the operator from the necessity of active control of the process is measured in terms of time saved and the elimination of errors to which we frail humans are subject.

Automatic regulators of the type that are commonly used when a single temperature is desired, function merely to hold the retort at the proper temperature during the major part of the process. A more complex type of controller for retorts has been developed which can control several, or all, of the manipulative operations of a process. Sampson (1930) and Schmid (1932b) describe a controller of this character which makes a process completely automatic. Short coming-up time, accurate control of the processing temperature, rapid cooling, and complete control of pressure throughout the process are assured. The only attention required is the turning of a key to start the process. These controllers have attained more extensive use in the processing of glass containers than in the processing of tin containers because of the greater complexity of the control problem in glass processing, according to an anonymous article in *Canning Age* (1932) and to White (1932). Cycle controllers have been used by the Big Stone Canning Company in processing No. 10 cans of whole-grain corn, however, since 1931.

From the standpoint of the effect of retort manipulation on the preservation of quality in canned foods, cycle control brings the "still" retort within a very short step of the continuous type of process kettle, which is the ideal equipment for time control of heat sterilizing processes for foods sealed in containers.

Continuous and Agitating Cookers

In continuous pressure cookers the sealed containers are taken into and removed from pressure chambers by means of valves that prevent the loss of pressure from the chambers. With temperature and pressure in all chambers controlled automatically, the continuous retort has all the advantages that are given to the "still" retort by cycle-

process control. In addition, it can operate so as to subject the container instantaneously to the holding temperature of the process and to terminate the heat treatment abruptly at the prescribed time. These features are advantageous in the processing of most canned foods.

Jarvis (1931) studied the processing of many products in an experimental continuous unit, adjustable for either agitating or non-agitating pressure cooking, and reached the conclusion that many foods are improved, over the same foods as ordinarily processed, by the use of a high temperature and a shorter process.

The usual accompaniment of continuous operation is agitation of the food during processing. Agitation is another important factor from the standpoint of preserving quality in the food. In some cases, it is a benefit; in other cases, it is not. For most foods that are benefited by agitation during processing, the benefit comes solely from the effect of agitation in accelerating heat penetration into the food. Agitation is a benefit to some foods, however, (for example, evaporated milk and potted meat) because of its effect on the physical appearance of the food. Certain foods, on the other hand, cannot stand agitation because of its detrimental effect on their physical appearance. Tender pieces of food may be broken up by agitation. Separation of the constituents of a food is another phenomenon that sometimes results, for example, agitation of cream-style corn during processing increases the tendency of the sauce to coagulate.

Seeing a need for a continuous cooker that does not agitate the cans, Anderson-Barngrover Manufacturing Company, in 1931, modified their regular cooker and placed on the market a machine in which the amount of agitation of the can was greatly reduced, as described in an anonymous article in *Canning Age* (1931).

The Thermal Engineering Corporation, Richmond, Virginia, manufactures a non-pressure continuous cooker in which the amount of agitation of the can is ingeniously controlled by means of an adjustable mechanism that rotates the can at a predetermined rate independent of the rate of speed at which the can progresses through the cooker. Aside from this agitation-control feature, the cooker is similar in principle to the Wonder Cooker which has been in use for many years.

There are also retorts designed for processing by the batch method, which provide agitation. Such retorts, in which the agitation is provided by rotation of a reel, are manufactured by Berlin-Chapman Company and Fort Wayne Dairy Equipment Company. In 1935, the Continental Can Company introduced a retort in which agitation is produced by reciprocal oscillation of the entire retort load.

An important influence on the effect of agitation on food in cans is the amount of head space in the cans. In all cases in which the agitation effect on the food is critical, it is essential that the amount of head space in the cans be uniform, and accurate control of this factor is necessary to obtain the desired effect.

The rate of heat penetration into some products is practically unaffected by agitation because agitation does not stir the product, that is, it does not move the solid particles of food about with respect to each other; examples of such products are roast beef, salmon, and squash. Although agitation may not adversely affect the quality of these products, no appreciable benefit is gained by agitating them during processing.

Pressure-processing machinery is like all other classes of mechanical equipment in that many of the types described in issued patents are impractical and unworkable. Nevertheless, a résumé of patents by Bitting (1916) does present an interesting story of the mechanical changes that have brought about the developments in processing technique that we have discussed. This résumé covers the period from 1869, the date of the first patent issued for a pressure cooker in this country (a retort in which steam was generated within the cooking chamber), to 1916. The spiral type of continuous-pressure cooker, which is extensively used today, was patented July 11, 1899.

We shall not discuss further the reasons for improved quality resulting from scientific retort manipulation, which brings about rapid rise of temperature in the retort, rapid cooling of the containers, and uniform treatment of the containers throughout the process. Discussion of the various phases of this subject will be found in the literature already cited, as well as in the writings of Bigelow (1922), Dillman (1932), Flannery (1929), Gammon (1922), Harrison (1933), and anonymous writers in *The Canner* (1929) and in *Food* (1936). On the subject of cooling alone there is a profusion of published information, most of which pertains to the technique of cooling containers under pressure. Among these publications are those of Chambellan, Cheftel, and Thuillot (1932), Continental Can Company (1934, 1936), Locke (1932), Vaughn (1929), Wiegand (1925), Baker (undated), and an anonymous writer in *Food Manufacture* (1935).

RELATION BETWEEN PROCESSING TEMPERATURE, HEAT PENETRATION, AND QUALITY

Foods that have a high rate of heat penetration, whether it be produced by convection currents in the food or by agitation of the food during heating, will usually have better quality after processing if they are sterilized at temperatures in the higher range, e. g., above

121.1°C.(250°F.), than they will if sterilized at a lower temperature. Conversely, articles having slow heat penetration will usually have better quality after processing if they are sterilized at a temperature below 121.1°C. Furthermore, foods having rapid heat penetration will usually have better quality after being sterilized at a high temperature than will foods having slow heat penetration after being sterilized at a low temperature.

PRINCIPLE OF HIGH-SHORT STERILIZATION

From these facts we are led to the conclusion that, by increasing the rate of heat penetration into a food that is impaired in quality by the ordinary sterilizing process, and by raising the processing temperature, we shall improve the quality of the finished product. We conclude also that, within limits, the amount of improvement will be in proportion to the increase in rate of heat penetration and the increase in temperature.

The rate of heat penetration of whole-grain corn, for example, is quite high, especially in the smaller cans. Whole-grain corn in No. 1 cans, better in quality than today's commercially canned corn, could probably be produced by processing at 148.9°C.(300°F.) instead of at the usual 115.6 or 121.1°C. Processing at 148.9°C. would be satisfactory if the retort or continuous cooker were made to withstand the high pressure required and the manipulation of the process were faultless.

We find ourselves now delving into the principle of "high-short" sterilization, of which there was prophetic utterance as far back as 1898, but toward the commercial use of which progress has been reasonably slow.

DEFINITION OF HIGH-SHORT STERILIZATION

High-short sterilization of foods is sterilization by heat applied for times ranging from a few seconds to a few minutes. High-short sterilization implies heat penetration so rapid that, to obtain it, the food must either be heated in bulk or in containers that are kept in violent agitation. Bulk heating may be done either by steam or hot water passing directly into the food or by indirect heating of the food in thin layers which are usually in agitation. In some high-short methods the food is heated after it is in the can but before the can is sealed. For nonacid foods a high processing temperature, e. g., 126.7°C.(260°F.), is necessary. This requirement is in accordance with the principle, previously expressed, that higher temperatures have less effect in quality impairment, in proportion to their lethal power, than lower temperatures have.

According to the procedure in heating, filling, and sterilizing food, high-short sterilization methods are classified as of five types:

1. Food heated but not completely sterilized before it is put into the can.
2. Food completely sterilized before it is put into the can.
3. Food heated in the can before sealing, with sterilization completed after sealing.
4. Food heated in the can and completely sterilized before sealing.
5. Food heated in the can after sealing.

Under Type 2 operation with non-acid food, unless the can is sterilized before it is filled and the cover is sterilized before it is sealed onto the can, the food must be at high temperature when the can is sealed; and it must be maintained at high temperature a sufficient length of time to sterilize the inside of the can. To accomplish this the food itself must be subjected to a double sterilization treatment. The first part of this treatment sterilizes the food before it is put into the can, and the second part sterilizes the can after it is sealed. If the double sterilization treatment of the food is to be avoided, the can and cover must be presterilized.

Under the other four types of operation, presterilization of the can and cover is not required.

RELATIVE ADVANTAGES OF HIGH-SHORT STERILIZATION METHODS

High-short sterilization by methods that require a portion of the sterilizing action to be exerted after the food is sealed in the container (Types 1, 3, and 5) has two disadvantages when compared with methods in which the food is completely sterilized before it is put into already sterile containers (Type 2) or in which the food is sterilized and cooled in the can before sealing (Type 4). One of these disadvantages lies in the fact that all cooling of the food after sterilization must take place in the sealed container, and with certain foods in large containers the cooling is so slow that preservation of quality to the full extent that should be possible in high-short sterilization cannot be realized. The other disadvantage lies in the mechanical difficulty of sealing the container under high pressure when the food is at a high temperature.

On the other hand, these methods have certain advantages over those in which sterilization of the food is completed before the food is put into the container. The equipment for treating the food prior to filling is simpler, the container and cover do not have to be sterilized before receiving the food, and the filling and sealing of the container do not have to take place under aseptic conditions.

EXPERIMENTAL WORK IN HIGH-SHORT STERILIZATION

Begun in 1927 and still in progress, our extensive experiments in high-short sterilization have included four of the five methods and many food products, including evaporated milk, whole milk, cream-style corn, whole-grain corn, peas, diced vegetables, lima beans, mushrooms, fruit juices, cream soups and puréed vegetables, cereals, and fruits. The method under Type 4 was not included in this study.

In a development project in which several types of technique are studied there is always a trend, or gravitational movement, toward use of the type of technique that has the greatest number of points in its favor. In our study, as equipment and technique have been improved, the natural trend has been toward the method by which the food is sterilized and cooled before it is put into the can. This method is applicable to practically all types of food. It provides the means of using a shorter heat treatment than any other method, and consequently produces the least change in color and flavor. We have packed all of the different foods by this method except those of discrete-particle type, such as peas, diced vegetables, and whole-grain corn, with which the Type 3 technique only has been used.

Nearly all of these foods, especially milk, corn, peas, and cream soups, after sterilization, are of outstanding quality—practically equal in color, flavor, and texture to the non-sterilized, or plain, home-cooked foods.

The major development in methods resulted from the work with evaporated milk. This product was packed by all four methods with the use of heating periods ranging from less than 30 seconds to more than five minutes, and with sterilizing temperatures ranging from 121.1 to 148.9°C. (250 to 300°F.).

Foods, such as meat and fish, that are packed in large, solid pieces are not adapted to high-short sterilization by methods in which the heat is applied to the food externally. The only methods of high-short sterilization that might be used successfully with such foods are those in which lethal energy can be generated within the food tissues, as by electrical heating (see the review of patents).

Our studies, in which more than a half million cans have been packed, have definitely established the applicability of the high-short sterilization principle to liqueform and discrete-particle form foods to produce improved quality. Sterile products are produced, most of which have greatly improved color and flavor as compared with the regular commercial products. The only questions remaining to be answered pertain to economic feasibility, mechanical practicability, and, in the case of some foods, the chemical and physical stability in storage, which affects the preservation of the quality that is present

immediately after sterilization. Prominent examples of foods with which the last problem is a factor are peas, milk, and fruit juices. These foods are inclined to deteriorate in quality with considerable rapidity during storage, owing to chemical or physical instability. Most of the other foods studied retain their excellent quality through a long period of storage.

COMMERCIAL USE OF HIGH-SHORT STERILIZATION

Acid Foods

In the United States high-short sterilization is applied in commercial canning only to fruit juices. It has, during the last five years, gained recognition as an indispensable adjunct to deaeration in the production of the finest quality in some of these articles. Marsh (1937) presented convincingly the reasons for popularity of the high-short method in this field.

The operation with fruit juices is under Types 1, 4, and 5. In Type 1 operation the juice is first heated and may be partially cooled before filling into cans that have not been presterilized, but it is filled at such a temperature that the can and cover will be sterilized by heat from the juice. The stored heat will also complete the sterilization of the juice itself, if it is not completed before filling. In Type 4 operation a can having a valve member in one end is used, such as that devised by Fenn, and discussed on page 38. In Type 5 operation any heating mechanism that agitates the can violently is used.

Nonacid Foods

Statements reach us from time to time claiming successful commercial high-short sterilization of milk in Europe. Recently, information was received that Carp and Company, Amsterdam, Holland, had developed a process for liquids based on improvements devised by van Zijderveld, Jr., and associates (British Patent 433,760); and that this process is being used commercially for milk at Woudenberg. The process is being operated without means for filling and closing the containers aseptically.

Writers have been penurious in referring to the application of high-short methods to the sterilization of nonacid food products. Perhaps the reason is that few persons except inventors and promoters have been keenly interested in the subject, and these, as a rule, are not inclined to publish what they know, except in patent form, until they have carried their processes beyond the purely experimental stage. Consequently, most printed matter pertaining to the subject of high-short sterilization of nonacid canned foods consists of either

patents or prospectus advertisements issued by promoters. A few prophetic statements can be found, however, in writings other than patents.

Nelson (1932) stated: "The 'flash' methods for sterilizing liquids probably most closely approach the ideal heating method and have been found to be very satisfactory for some products. The disadvantage of the methods is that the sterilized product must be filled into sterile containers without being subjected to recontamination by spoilage organisms." Nelson therein stated the principal reason for our not yet having high-short sterilization of nonacid foods, although we have it for acid foods. Although it is not necessarily true that the food must be filled into sterile containers, the difficult part of the mechanical problem is present no matter what type of operation is followed. This difficult feature is the filling and sealing of the containers under pressure greater than that of the atmosphere. This is necessary either to prevent boiling of the food when filled at high temperature or to assure that filling and sealing be done under aseptic conditions.

TYPE 1 HIGH SHORT STERILIZATION

Ball (1926) stated that if cans could be filled and hermetically sealed at a temperature of 121.1°C. (250°F.), or higher, and a few minutes consumed in conveying the cans under proper conditions to the cooling tank, the contents of the cans would be sterile by the time cooling was begun. Canadian Patent 285,317 (1928) describes a process involving this principle. The method comprises heating the product in bulk in a closed chamber to a temperature above the boiling point of the liquid content of the product at atmospheric pressure; depositing the product while hot into a container while under pressure to prevent boiling; closing the open end of the container while under pressure; turning the container to sterilize all of its inside surfaces by the heat of the product while still at sterilizing temperature and while the sterilization of the product is being completed, and cooling the filled and sealed container under controlled pressure conditions. This exemplifies the application of the principles of Type 1 operation, under which the food is heated but not completely sterilized before it is put into the can.

TYPE 2 HIGH SHORT STERILIZATION

Cans Processed After Sealing

The technical branch of the canning industry seems to have found **most** of its attraction to the field of high-short sterilization in the **procedure** for completely sterilizing the food in bulk and then putting it into sterile containers. The problem of filling and closing the con-

tainers aseptically has been the primary source of worry to originators of these methods. In more than half of the score of patents, it is either stated or assumed that bacterial contamination of the food will take place during filling and sealing of the containers, and provision is made for subjecting the containers to a mild sterilizing process after they are sealed.

Cuykendall: Cuykendall invented a continuous pressure cooker for corn, U. S. Patent 695,196 (1902). The patent does not reveal the method of applying this cooker to canning under the principle of high-short sterilization. I am told that when such application was made experimentally the sealed container was given a process in boiling water which was expected to destroy not only the bacteria that got into the corn during filling and before closing but also bacteria that were on the inside surfaces of the container at the time of filling. The corn was heated in bulk by applying steam directly into the corn under pressure and was then cooled to a temperature of about 82.2°C. (180°F.) before filling.

Mollinger: A process was applied by Mollinger, U. S. Patent 1,005,275 (1911), to fluid milk. In a period of two seconds, he heated milk to a temperature of from 130 to 155°C. (266 to 311°F.) while it was flowing through steam-heated tubes, cooled the milk immediately to about 98°C. (208.4°F.) while the milk was passing through tubes, filled and sealed the container while the milk was at this temperature, and cooled the container very slowly to about 70°C. (158°F.). The final prolonged cooling operation served the purpose of a process of the sealed container to destroy bacteria that may have entered the milk accidentally during the filling and sealing operations.

Grindrod: Proceeding from an emulsifying process in which high-velocity steam jets were used, U. S. Patents 1,435,464 (1922) and 1,461,653 (1923), Grindrod developed a high-short sterilizing process for liqueform products. In this process, U. S. Patent 1,714,597 (1929) and Reissue 19,193 (1934), the food, in bulk, is heated by steam in high-velocity jets. When sterilization is completed, the food is condensed by evaporation to the desired proportions. The temperature effect itself is said to be assisted in destroying the bacteria by direct impact upon the bacterial cells of particles of steam moving at high velocity. The material is cooled rapidly as it enters the evaporating chamber. In the opinion of the inventor, sterilization is assisted also by the high rate of change in temperature of the food, which rate is said to be beyond the adaptive power of the microorganisms.

Means for canning is not provided, and it is expected that in the canning operation air-borne bacteria of low heat resistance will find their way into the food. The suggestion is made that to destroy these

bacteria the sealed can be placed in a sterilizer of suitable form, such as that used for sterilizing milk, and given a heat treatment of about ten minutes at 110°C.(230°F.).

U. S. Patent 1,797,769 (1931) described an apparatus having a special type of nozzle which delivered sterilizing fluid into the food in multiple jets along conically divergent lines.

The process was improved by the introduction of more positively controlled action to increase the efficiency of the impact, friction, and rapid change in temperature effects, U. S. Patent 1,819,023 (1931). Apparatus was adapted to the improved technique to give 'direct single or simultaneous impact' of the high-velocity steam on the food and bacterial cells, U. S. Patent 2,019,491 (1935), and to better adapt the process to continuous operation with large output, U. S. Patent 2,020,309 (1935).

Grindrod recommended the process for the stabilization of liquefied food materials, such as milk substances, to produce a jelly formation in the finished product, U. S. Patents 1,798,120 (1931) and Reissue 19,868 (1936); and against coagulation, U. S. Patent 1,854,189 (1932); as well as for the flash sterilization of fruit juice under absolute pressure of from three to 14 pounds per square inch after the juice had been preheated to 48.9°C.(120°F.) in a heat exchange apparatus and deaerated, U. S. Patents 2,059,788-9 (1936).

The process has had commercial application in the stabilization of colloidal substances and in the sterilization and stabilization of canned dairy products, such as chocolate milk drinks. The process is handicapped, however, by its lack of an aseptic filling and closing means.

Hucker and Hucker (1929) investigated the Grindrod process with regard to milk, and they believe that the process may be applied successfully to the sterilization of milk products.

Rafn: A European inventor, William Rafn, designed a process for milk and other or similar food products, U. S. Patent 2,049,591 (1936), in which the food is subjected to the sterilizing effects of a temperature above 120°C.(248°F.) for a period of time not exceeding one minute in a closed heating chamber through a continuous process and thereupon cooled instantaneously. The food is then filled into sterilized containers which are sealed and then passed through a sterilizing treatment at a temperature between 100 and 107°C.(212 and 224.6°F.) for a period of approximately eight minutes.

Sealed Cans Not Processed

Although inventions of high-short sterilization methods are still being produced, providing for the filling and closing of sterile food in containers under non-aseptic conditions, there is evidence that the

importance of aseptic filling and closing was recognized at the beginning of the era of inventions in this field. Starting with the dawn of the present century, numerous patents have been issued on methods that do not require processing of the containers after sealing.

Plummer and Stare: For describing the virtues of high-short sterilization, it is hard to find a better statement than that of Plummer and Stare, U. S. Patent 699,765 (1902). The actual provisions of their patent would point to the classification of their method in Type 1, under which the food is put into the containers before it is sterile. The specification refers, however, to the thorough sterilization of the material preparatory to its insertion into the can. The handling of the sealed container is not covered.

Dunkley: Dunkley's utilization of high-short sterilization technique, U. S. Patent 1,270,797 (1918), was a distinct scientific retrogression from the disclosure of Plummer and Stare. His operations were identical with those of Plummer and Stare except that his canning chambers were open to the outside atmosphere and were filled with steam at atmospheric pressure. This made the method impractical for nonacid products, although it was entirely practical for acid food materials.

Hansen: Patents describing the various high-short sterilization methods of Hansen are characterized by their emphasis on the technique of heating the food material. U. S. Patent 1,696,399 (1928) discloses a method of heating food material of discrete-particle nature, such as fruit berries, by rolling the berries over successive surfaces confined within a succession of chambers and applying gradually increasing temperatures to the berries, and subsequently either cooling or immersing them in hot syrup. Another method of treating foods of discrete-particle nature, U. S. Patent 1,756,549 (1930), is to heat the solids in the absence of liquid, either in bulk or in the can, to sterilize the solids and then to cool the material by immersing it in a sterile liquid. The solid material may be partially cooled before it is immersed in the liquid. A third method of treating this type of food, U. S. Patent 1,756,550 (1930), is to heat it either in bulk or in the can, by immersing it in hot liquid sufficient to sterilize the food. In the last two instances, it is provided that the final sterile mixture may be sealed in containers under sterile conditions.

These methods were improved, U. S. Patents 1,857,450 (1932) and 2,011,631 (1935), by the addition of the use of pressure in the chambers in which the food is treated. In the improved process, discrete-particle solids, such as peas, are sterilized by heat from a liquid that is mixed with the granular material. After a specified time the heating liquid is removed from the mixture, and the solids are submerged

in other sterile liquid. These operations are carried out under pressure. Canning then proceeds under aseptic conditions. The second portion of the liquid used in the process may be either cool or hot when it is mixed with the hot solids, depending upon whether or not the sterilization of the material has been completed by the time the liquid is added. The food may be put into the cans before treatment is begun, if this seems desirable. Thus the process may be classified as of either Type 2 or Type 4. As in the Grindrod method, there is no provision in regard to handling the containers after sealing.

Another method of sterilizing liqueform food material, U. S. Patent 1,935,777 (1933), is by the application of heat conducted through material with the surface of which the liquid is in contact while it is being transported through the apparatus. The device is so constructed that the contact surfaces are automatically kept clean by wiping. A canning means is not provided.

McClatchie: McClatchie proposed a process for evaporated milk, U. S. Patent 1,821,943 (1931), in which the operations were essentially the same as those of the Grindrod process except that in the sterilizing chamber McClatchie sprayed the milk into a steam atmosphere, whereas Grindrod sprayed steam in high-velocity jets into the milk. After having been sterilized the milk was reduced in temperature and condensed in an evaporating chamber. It was then canned, presumably in sterile containers, although no means was provided for sterilizing the cans. Differing from Grindrod, McClatchie claimed no effect other than temperature effect in sterilizing the milk.

Heyman: Heyman disclosed a method, U. S. Patent 1,842,969 (1932), almost identical with that of Plummer and Stare, U. S. Patent 699,765, with the addition of steps which have a bearing on the quality of some types of finished product but which are not important from the sterilization angle.

Van Zijderveld et al.: Van Zijderveld and associates have a process, British Patent 433,760 (1935), in which, by virtue of the use of a specially constructed tubular apparatus, liquid substances are agitated violently while they are being heated, sterilized, and cooled in passing through the tubes. Means for aseptic filling and closing of containers is not provided.

Ball: A process by Ball, U. S. Patent 2,029,303 (1936), provides for the operations of sterilizing empty containers, sterilizing and cooling covers for the containers, sterilizing a food material in bulk, putting the sterilized food into the sterilized containers, and applying the sterilized covers to seal the containers. All operations in handling the sterilized objects until after the containers are sealed are performed under aseptic conditions, which are maintained in closed chambers by

the presence of steam under a pressure greater than that of the outside atmosphere.

Chapman: An apparatus was devised by Chapman, U. S. Patent 2,049,639 (1936), for heating food of discrete-particle type while the food was in mixture with liquid and flowing continuously. After being sterilized the food particles were separated from the liquid. This device was improved, U. S. Patent 2,057,366 (1936), by the introduction of means for accurately controlling the thermal treatment and permitting the use of temperature above the boiling point of the liquid at atmospheric pressure. Means are also provided for cooling the food and releasing it into atmospheric pressure without damage to the food.

TYPE 3 HIGH-SHORT STERILIZATION

It may seem strange that inventors have so generally neglected high-short sterilization methods in which the article of food is put into the can and is then heated in the unsealed can to receive a store of sterilizing heat, so to speak, after which the can, containing the food at high temperature, is sealed under pressure and then held before cooling a sufficient length of time to complete sterilization of the food material and container. Numerous methods have been devised which follow this procedure up to the point of sealing the container but which provide no means for insuring the production of a sterile product in case the food and the container are not already sterile at the time of sealing.

Ball and Wilbur: A method is described by Ball and Wilbur, applying to products of discrete-particle nature, U. S. Patent 2,040,726 (1936). Open-top cans, containing the solid particles without free liquid, are passed in inverted position through a chamber containing steam of high temperature and pressure, while steam is circulated among the particles. The cans are in the inverted position to prevent the accumulation of liquid in them and to facilitate the removal of air and the circulation of steam among the food particles. After the food throughout has reached the temperature of steam in the chamber, the containers are sealed while still in steam under pressure; and the sealed containers are further subjected to an atmosphere of steam to prevent loss of heat from them until sterilization of both food and containers has been accomplished. The containers are then cooled under controlled pressure conditions.

Ayers and Lang: A can having a specially constructed valve member is used in a process designed by Ayers and Lang, U. S. Patent 2,054,065 (1936) and French Patent 343,176 (1934), for treating milk products. After the food is confined within the can, the can is vacuumized through the valve opening. Steam under pressure is then forced

into the can in such a way as to pass through the food article, agitate it, and heat it to a temperature within the limits of approximately 115.6 and 137.8°C. (240 and 280°F.). The can is then sealed by closing the valve permanently, and the sealed can is held, before cooling, a sufficient length of time to insure commercial sterility of the contents. In order to insure proper proportion of solid to liquid material in the finished article, the inventors give specifications for preparation of the food for the process, as well as for manipulation of the process.

TYPE 4 HIGH-SHORT STERILIZATION

The type of high-short sterilization technique in which both heating and complete sterilization of the food take place in the can before it is sealed, stands second in respect to the amount of attention received from inventors. The processes of this type may be divided into two classes, namely, those in which the food is not cooled before the can is sealed and those in which the food is cooled before the can is sealed.

Food Not Cooled Before Sealing

In four of the five processes belonging to this fourth general type, the container is sealed while the food therein is still at the highest temperature to which it was heated for sterilizing. Thus there is still potential sterilizing energy in the sealed cans; but in the absence of means to control its effect, it cannot be utilized. Sterilizing effect after the container is sealed, therefore, is not included in defining these processes. Inasmuch as the manner of handling the container after sealing is not prescribed, the processes can insure production of sterile products only when both food and container are sterile at the time the container is sealed.

Fenn: Fenn described a process in which the food material in the unsealed can was sterilized by injection of steam or other hot vapor or gas while the can was enclosed in a sterile chamber under pressure. The container was then sealed in the sterile chamber under pressure. In the first application of the process, U. S. Patent 1,141,242 (1915), the steam was discharged from a nozzle inserted into the food in the open-top container, and the cover was sterilized by steam in the sterile chamber before the cover was put onto the container. It was preferred that the emission of steam from the nozzle be intermittent. In the second application of the process, U. S. Patent 1,365,673 (1921), steam was permitted to enter the food in the container through a loose joint between cover and container when the container was up-ended. The cover, like the side walls and bottom of the container, was sterilized by heat from the steam that entered the container. The third application of the process, U. S. Patent 1,378,531 (1921), differed from the

second in details of the sealing operation and in the fact that provision was made for exhausting the steam from the chamber slowly to prevent food material from boiling out of the can before it was completely sealed.

Later, U. S. Patent 1,563,971 (1925), it was found desirable, in connection with the second and third applications of the process, to incorporate the preliminary step of vacuumizing the container to permit the steam to produce the rapid heating of the food that was desirable.

To facilitate the operation of the process, a container was developed having in one end a specially constructed valve member, U. S. Patent 1,732,227 (1929), which would provide an opening for vacuumization and steam injection and which could be closed by a simple operation to permanently seal the container. A machine was designed for processing food in the valved container in a continuous manner, U. S. Patent 1,938,821 (1933). In this machine the container is inverted after the vacuumizing operation so that the steam, after entering the container through the valve member, passes upward through the food. The container is again inverted before sealing. The inside surfaces of the container are made sterile, along with the food, by heat from the steam that enters the container.

For several years this process has had commercial use in the United States in sterilizing citrus juices.

Hansen: A process, designed by Hansen, for the primary purpose of removing air from fruit during canning is mentioned because it is a high-short sterilization process, U. S. Patent 1,625,207 (1927). All the operations, which take place after the fruit is in the container, are as follows: (1) exhausting the air; (2) heating the fruit, preferably by admitting steam or a hot, inert gas; (3) submerging the fruit in a liquid; and (4) sealing the container. A final sterilizing operation may be used after the liquid is put on the fruit.

Kronquest: A process resembling the original process of Fenn was invented by Kronquest, U. S. Patent 1,863,447 (1932). The filled, open-end container, enclosed within a pressure chamber, was treated with steam issuing from a nozzle inserted into the food. After the steam treatment, the container was hermetically sealed while still in the chamber under pressure.

Loetscher: Loetscher patented a process, U. S. Patent 2,060,736 (1936), differing from that of Kronquest in one respect. The can of food was held with the open end down during treatment with steam in the Loetscher process, so that liquid was drained from the can. By this procedure the process was limited in its application to products of the separate-particle type.

Food Cooled Before Sealing

When the food is cooled after sterilization in the can before the can is sealed, the pressure required in the sealing chamber to protect the food from bacterial contamination is lower than when the food has its maximum sterilizing temperature at the time of sealing.

Hansen: A patent of Hansen, U. S. Patent 1,390,703 (1921), devotes itself to a technique of sterilizing food in open containers. The procedure consists of transporting containers filled with food through segregated successive zones of gradually increasing temperature, and subsequently transporting the containers through segregated successive zones of gradually decreasing temperature. Means for handling the containers thereafter is not considered.

TYPE 5 HIGH-SHORT STERILIZATION

As previously stated, in order to have high-short sterilization by heat applied to food after it is sealed in a container, one must subject the container to a high processing temperature and must agitate the container violently during the heat treatment. High-short processes of this type must be of longer duration, on the average, than processes in which the food is heated in bulk before it is put into the cans or is heated in the open can. I believe we are justified in stating arbitrarily, however, that a process in which the heat treatment is longer than six minutes is not a high-short sterilization process.

Bonine: For sterilizing milk, Bonine disclosed a process, U. S. Patent 1,081,483 (1913), in which the sealed container, filled with milk to approximately 90 per cent of its capacity, was agitated violently while it was heated to about 130°C. (266°F.). The container was then cooled while still in agitation. Cooling in an air blast was suggested.

Hansen: Though perhaps not a high-short sterilization process according to our arbitrary definition, an agitated can process of Hansen is mentioned because of its disclosure of a principle, U. S. Patent 1,474,820 (1923). The food-laden, sealed container is subjected to a temperature not in excess of 115.6°C. (240°F.), with or without moderate agitation to cook the food, and is subsequently subjected to a temperature of at least 121.1°C. (250°F.) under violent agitation for a short time to sterilize the food. The inventor recommended that the agitation be produced by imparting a reciprocating movement to the container.

Rafn: Rafn of Norway designed a process for sterilizing liquids in sealed containers, British Patent 208,140 (1924). It is suggested that the containers be heated to a temperature within the approximate limits of from 130 to 140°C. (266 to 284°F.). During heating the con-

tainers are subjected to a special type of agitation designed to promote rapid transmission of heat into the food and to cause the liquid to wash the interior surfaces of the container energetically.

Chapman: A can agitator for producing a reciprocal movement of cans within a retort was designed by Chapman, U. S. Patent 2,050,560 (1936). The cans are confined within trays that are held in crates, which are moved in reciprocating manner and may be revolved about an axis at the same time.

Kennedy: In a process of sealed cans by Kennedy, U. S. Patent 2,056,526 (1936), each can is rotated about its longitudinal axis (as in rolling) while it is passed through a plurality of heating and cooling zones, one of which is maintained at a high critical temperature. For the heat treatment and cooling, a total time of about eight and one-half minutes is suggested when the process is applied to evaporated milk.

Page and Taylor: A process by Page and Taylor, U. S. Patent 2,062,331 (1936), involves an efficient method of agitation. The procedure is to agitate the food by rotating the sealed container on its longitudinal axis at the same time imparting a reciprocal movement in the direction of the longitudinal axis while the container is being subjected to heating and cooling media successively. Periodical reversal of the sense of rotation of the containers may be practiced in order to effectuate agitation of the food.

Ball: In final consideration of high-short sterilization processes employing steam as a heating medium, we look at a process by Ball. U. S. Patent 2,087,962 (1937). The process is applied to evaporated milk having total-solids content of over 29 per cent. The milk in sealed containers, kept in violent agitation, is heated at a temperature above 123.9°C. (255°F.) for a period ranging from three to 15 minutes and cooled. Proper combination of sterilizing temperature and time, and of degrees of evaporation and agitation are employed to control the viscosity of the finished product.

ELECTRICAL STERILIZATION

The idea of sterilizing canned foods by means of electricity has enticed investigators for many years. Even by the child, electricity is known as something that acts instantaneously; therefore, it appears to be the ideal medium through which to accomplish high-short sterilization.

Electrical waves of certain frequencies are known to have the property of specific lethal action on bacteria and there have been unproved claims of this property for waves of other frequencies. Such a claim has been made for electromagnetic waves of extremely high

frequencies, ranging from 10 to 150 megacycles per second, emitted by a vacuum-tube oscillator. Consequently, it has been thought that these waves would be effective in sterilizing canned foods.

Christie and Loomis (1929) found no evidence to support the theory that waves of these frequencies have a specific action on living cells. They found that the effect produced on animals can be fully explained on the basis of the heat generated by high-frequency currents which are induced in them. Other reliable evidence indicates that, at least until important new developments have taken place in the electrical field, if we are going to have electrical sterilization of food materials, we shall have to place our primary dependence upon the old, staid, and tried heat effect to produce results, just as we do in steam sterilization.

With mere mention of the fact that investigations have been made of the possibility of sterilizing food by inductive effect in the field of high-frequency oscillating currents, we shall take up the subject of producing heat in food by resistance to electrical current passing through the food.

Electrical Heating of Food in Bulk

In the high-short sterilization processes already discussed under Types 1 and 2 of the methods employing steam or water heating, it should be feasible and practical to heat the food in bulk electrically by applying the principle of the process now operating in the commercial pasteurization of milk under the name of Electropure. By the Electropure process the milk is heated as it flows between two carbon electrodes, by an alternating current passing through it. In this pasteurizing process, milk is heated to a temperature of 71.1°C. (160°F.) in from 20 to 30 seconds, and it is held at that temperature for about 15 seconds.

Gelpi and Devereux (1930) found that the Electropure process produced from 71.5 to 99.9 per cent destruction of typical bacterial endospores, whereas the ordinary holding method of pasteurization at 62.8°C. (145°F.) produced only from nil to 13 per cent destruction. Other similar results are available to indicate the possibilities for electrical heating in high-short sterilization.

Jones: An apparatus for heating liquid with an electrical current while the liquid is in continuous flow was invented by Jones, U. S. Patent 592,735 (1897). He employed an open container having equidistant sinusoidal electrically conducting sides; bottom and ends were composed of nonconducting material. The liquid was caused to flow from one end of the container to the other while an electrical current passed through it between the two sides which functioned as electrodes.

Anglim: The pasteurization of milk by a batch method was carried out by Anglim, U. S. Patent 1,468,871 (1923). The pasteurizing receptacle was equipped with spaced electrodes and an agitator. Agitation kept the milk moving between the electrodes while electric current passed through the milk. Thermostatic control was provided.

An apparatus in which the wall of the receptacle functions as an electrode is described by Anglim in U. S. Patent 1,984,956 (1934). The other electrode was centrally located in the receptacle. An agitator was provided.

Electrical Heating of Food in the Can

The sterilization of food in a can by means of an electric current is an appealing thought, but the problems encountered in finding a practical way to pass the current through food in the can are many and formidable. The canning industry in this country was not far advanced when the first courageous inventor ventured to show the industry a container adapted to this operation; and not until 25 years later did another publish his idea of how the job should be done.

Roberts: Before the 20th century was three months old Roberts held a patent, U. S. Patent 645,569 (1900), on a container composed of a conducting material and provided with an interior insulated electrode with a connector extending therefrom through the wall of the container. The container was intended for foods that would be sterilized by an alternating current which would pass through the food between the insulated electrode and the container walls. The container would constitute the other electrode. A plate or cap was provided for sealing the container at the point at which the insulated electrode entered after the sterilizing operation was completed.

Hull: Hull employed an arrangement of container and central insulated electrode, U. S. Patent 1,522,188 (1925), similar to that of Roberts, but provision was not made by Hull for sealing the sterilized food in the container. For heating emulsions, such as milk, alternating current of high frequency was used to prevent the deposition of solid material upon the electrodes.

Bohart: Neither Roberts nor Hull provided a means for distributing the flow of current through the food in his container so as to give effective results. Furthermore, he apparently failed to realize that heat was the sole lethal agent in electrical sterilization by his method. Bohart, on the other hand, took both of these factors into consideration in designing his container, U. S. Patent 1,911,879 (1933). He actually determined the rate of increase of temperature in cream-style corn in his container while passing through the corn an alternating electrical current of 60 cycles, 7 amperes, and 110 volts. The temperature was raised from 83.3 to 121.1°C. (182 to 250°F.)

in approximately $1\frac{3}{4}$ minutes; and after holding the corn at 121.1°C . for a certain length of time to sterilize it, he had a product greatly superior in color and flavor to corn processed in the can in the usual manner.

In Bohart's container the entire body portion, to which the two ends are attached, is of electrically nonconductive material, and the two ends which are sealed onto the body portion are formed wholly or in part of an electrically conductive material. The electrical current flows through the food between the two ends of the container, which function as electrodes.

U. S. Patent 1,961,681 (1934) covers the method applied by Bohart to nonliquid food, which comprises preheating the food to substantially 82.2°C . (180°F .), hermetically sealing it in a container, and then passing an electrical current through it to heat it until substantially 121.1°C . (250°F .) is reached in the center, and reducing the current to maintain such temperature for approximately 10 minutes.

Steerup: Another special container, intended to function in a similar manner to that of Bohart, was designed by Steerup, U. S. Patent 2,013,675 (1935). All three members—body and both ends—are of metal. In the sealing joint between the body and one end there is electrical contact between the members. In the sealing joint between the body and the other end electrical contact between the two members is prevented by the presence of a fibrous liner of electrically insulating material. Fibrous insulating material, similar to and perhaps integral with that in the joint between the end and the body of the container, covers the interior surface of the body member. The purpose of this insulating liner on the body of the container is to force the electrical current to pass completely through the food from one end of the container to the other.

Ball: A method of applying this principle of electrical heating to the food in a container before it is sealed is described by Ball, U. S. Patent 2,091,263 (1937). Any type of container having a sufficiently large opening in the top is suitable for use in this process. The patent covers both method and apparatus. The procedure in the process is as follows:

An open-top can, containing food material, is passed into a pressure chamber. Electrodes are inserted vertically into the food in the container, and electrical current is passed through the food between the electrodes until the food has been brought to the desired sterilizing temperature. The container is then sealed while still under pressure and is turned to bring its longitudinal axis into approximately horizontal position so as to bring the hot food material into contact with all inside surfaces of the container, thus sterilizing these surfaces

while the food is still at sterilizing temperature and while the sterilization of the product is being completed. The container is then cooled under controlled pressure conditions.

PRESERVATION WITHOUT HEAT

At the outset of this exposition we named five agents other than heat which have been available to man from time almost immemorial, for use in the preservation of food materials. Improvements in the methods of utilizing these agents have developed into modern processes, some of which are bringing to us preserved foods of superior quality. The mirage of a miraculous process that will preserve food unchanged in character from that of the food in its original raw state, however, has led many a hopeful investigator into the promulgation of fantastic ideas which spelled grief both to the promulgator and to the gullible processor.

Numerous preserving processes in which the heat factor either is entirely lacking or is a subordinate factor are set forth in patents of comparatively recent issue. In order to give an idea of what has been covered in this field, we shall mention some of the patents.

Preservation by Freezing

Freezing processes deserve first mention because of their extensive application today in furnishing the food consumer with many varieties of products of superior quality. Advanced processing methods and apparatus for preserving foods by freezing are described in U. S. patents of Birdseye; for example, 1,773,079 (1930), 1,773,080 (1930), 1,773,081 (1930), 1,775,549 (1930), 1,977,373 (1934), and 1,998,431 (1935).

Preservation by Desiccation

Modern application of the art of desiccation in preserving food is well illustrated in the practice of powdering milk and similar substances. U. S. patents pertaining to this practice have been granted to Passburg, 1,049,141 (1912); Bunnell, 1,141,102 (1915) and 1,149,625 (1915); Campbell, Reissue 14,567 (1918); Heath, 1,406,381 (1922); McLaughlin, 1,616,631 (1927); Peterson, 1,748,618 (1930); Overton, 1,853,451 (1932); and Fechner, 2,065,675-6 (1936).

Chemical Preservation

In the class of chemical preservatives we include all chemical substances that are put into food for the purpose of preserving it whether the action of these substances is inhibitive or bactericidal. Patents disclose a variety of processes that function through the preservative action of substances added to foods. These substances may be in the form of gas, liquid, solid, or ions in solution. We shall mention some that are illustrative of the group.

Gas Substances: In U. S. Patent 1,141,238 (1915) Fenn described a preserving process that was carried out through the operations of vacuumizing food in an open container, treating it with a germicidal gas such as that obtained by heating solid or liquid formaldehyde, flushing out the germicidal gas, vacuumizing, and sealing the container aseptically.

Two years later, Franks, U. S. Patent 1,232,271 (1917), patented a process of which the procedure was to treat food in an open container with inert gases, such as carbon dioxide and nitrogen, under pressure of between 60 and 70 pounds per square inch, release the pressure, and seal the container in the absence of air.

Arnoldi found that the results of carbon dioxide treatment of milk were better when this treatment followed a removal of foreign flavors and gases from the milk by vacuumizing, U. S. Patent 1,403,223 (1922). The vacuumization was carried out with the milk in agitation at 37.8°C.(100°F.). The milk was cooled to 4.4°C.(40°F.) and then received the carbon dioxide treatment at a pressure of 60 pounds per square inch. Packaging was done under carbon dioxide pressure of 60 pounds.

A process for sterilizing food in liquid form while the food was flowing through a special type of apparatus was described by Frank in U. S. Patent 1,887,297 (1932). Carbon dioxide gas under pressure was discharged from a foraminous pipe so that it passed through the liquid, and the liquid was then passed through a suitable filter.

Hofius, German Patent 613,707 (1935), was able to increase the storage life of either raw or pasteurized milk to several months by a process consisting of cooling the milk to 6.1°C.(43°F.), shaking it to expel some of the air and other gases it contained, treating it with oxygen under pressure of 150 pounds per square inch, and storing at 6.1°C. under this pressure of oxygen gas.

Liquid Substances: The principal of asepsis, as it occurs naturally in citrus fruits, is the basis of a process of Kokatnur, U. S. Patent 1,715,932 (1929). A water extract of the skin of the fruit, which is said to contain a preservative substance, is added to the juice, after which the mixture is heated at a temperature of about 50°C.(122°F.) for at least two and one-half hours.

Metallic Ions: Preserving foods by utilization of the oligodynamic property of certain metals, such as silver and copper, has been studied by European investigators. Methods of applying these principles to foods are described in patents issued to Krause of Germany, U. S. Patent 2,028,072 (1936), and to Matzka of England, U. S. Patents 1,812,105 (1931), 1,813,064 (1931), and 1,850,594 (1932). These processes utilize a combination of moderate heating and contact of the

food material with the oligodynamically active substance. The food is usually contacted with two metals which are as widely separated as practicable in the electromotive series, and conditions are maintained to establish an electromotive force in the food, produced by difference of potential between the two metals.

Matzka provides for moderately heating the food material after it is sealed in the container in order to destroy bacteria with which the food may have become contaminated while it was being filled and sealed.

NOTE—In this paper, patents are described according to their claims, and not according to their specifications. In some, of course, there are disclosures in the specifications that are not included in the claims.

SUMMARY

Food preservation is treated historically according to its use in two periods, or epochs, viz., before Appert and after Appert, separated by the short period of Nicolas Appert's work, which is regarded as the most important period in canning history from a development standpoint. This period brought the inception of sterilization by heat combined with artificial asepsis through the sealing of food in containers.

Before the time of Appert humanity depended on fermentation, dehydration, addition of chemical substances, low temperature, and natural asepsis for the preservation of food. Since the time of Appert heat has had a constantly increasing application as a sterilizing means in food preservation. Although the other agents of preservation continue to be used and the methods of their application are constantly being improved, heat assumed first position in respect to extent of use among artificial preserving means many years ago.

In regard to the application of methods of preservation, foods are naturally classified according to their degree of acidity. Heat is used in the preservation of all types of food in this classification, but the methods of application differ according to the type of food. Non-acid foods, such as vegetables, meat, and marine products, present the greatest problems in this regard because of the extreme intensity of the treatment required to sterilize them. The requirements are so severe for some foods that the treatment impairs their appearance and flavor.

The development and improvement of equipment for heat processing canned foods comprises an interesting story. The design and manipulation of retorts have been improved along lines such as to permit the effective sterilization of nonacid products with less impairment of their quality than was previously possible. These improvements have been dictated by increasing knowledge of principles that form the

basis of heat sterilization. This knowledge is being revealed in the results of scientific study, which has increased greatly in intensity during the last quarter century.

One of the principles that have been learned is that the production of most retort-processed foods, having color and flavor which are most nearly like those of home-cooked foods, requires the retort operation to be such that (1) every container receives the same treatment, (2) the retort is heated rapidly to its holding temperature, and (3) the containers are cooled rapidly. These conditions have been promoted by the introduction of continuous and agitating types of retorts and by changes in design and the use of automatic controllers to facilitate improved manipulation of "still"-type retorts.

A further improvement of quality of nonacid canned foods, not yet an accomplished fact commercially, is expected to be realized from high-short heat processing. The value of high-short sterilization lies in its utilization of the principles that the higher temperatures have less effect upon food, in quality impairment, in proportion to their lethal effect than the lower temperatures have; and that quality in color and flavor is conserved by heating the food rapidly to the sterilizing temperature and cooling the food rapidly after sterilization.

Results of extensive experiments in high-short sterilization of evaporated milk show that heating at 115.6 and 143.3°C. (240 and 290°F.), respectively, produces an equal effect upon color and flavor when the heating at 143.3°C. has 4.4 times as great a sterilizing value as the heating at 115.6°C. Experiments with corn, peas, lima beans, mushrooms, diced vegetables, fruit juices, cream soups, and puréed vegetables, cereals, and fruits have definitely established the applicability of the high-short sterilization principle to liqueform and discrete-particle-form foods to produce improved quality. Some questions pertaining to economic feasibility, mechanical practicability, and, in the case of some foods, the chemical and physical stability in storage remain to be answered.

A comprehensive view of the present status of high-short sterilization by heat is provided in a review of 60 patents. Twenty-six additional patents are mentioned pertaining to food preservation by means of other agents than heat.

A bibliography of literature on the general subject of heat processing of canned food also is given.

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ACTION OF ENZYMES AT LOW TEMPERATURES¹

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It is evident that investigation of some of the better described enzymic processes at unusually low-temperature levels may extend knowledge of the mechanism of enzyme action on the one hand, and knowledge of tissue autolysis, with its practical implications for the cold-storage industry, on the other. Our work on the action of enzymes at low temperatures was started only recently under the Bankhead-Jones law providing for fundamental research on problems related to agriculture. The results presented in this paper are taken from a large amount of preliminary work conducted in this laboratory primarily for purposes of orientation. In the sense that further work is obviously indicated along certain lines, the results are incomplete; nevertheless, some of the observations are of decidedly more than passing interest and are accordingly presented.

The practical experience of industries depending upon the cold storage of foodstuffs has long pointed to the conclusion that enzyme action may occur even at very low temperatures. The work of Pennington (1912) has, in particular, demonstrated such effects in raw materials. The autolytic decomposition of agricultural products stored at low temperatures appears to be a major factor in their deterioration, and in some cases the only factor. The interpretation of such observations is, however, complicated by the presence of both enzymes and substrates as naturally occurring mixtures of very great complexity, and by the further circumstance that tissue autolysis is obviously faster in tissues once frozen and subsequently thawed than in tissues which have never been frozen.

One important simplification of the problem, so far as protein decomposition is concerned, is now possible by the use of the crystalline proteinases isolated by Northrop (1930), Northrop and Kunitz (1932a), and Kunitz and Northrop (1935) on a substrate, such as casein, crystalline egg albumin, or hemoglobin, that can be duplicated with considerable probability. Crystalline carboxypolypeptidase isolated by Anson (1935) may be used on simple substrates, such as chloracetyltyrosine.

STABILITY OF PROTEOLYTIC ENZYMES AT LOW TEMPERATURES

The investigation of enzyme action at low temperatures presupposes that the enzyme itself is not destroyed under such conditions.

¹ Food Research Division Contribution No. 246.

This question has been considered by many earlier workers, and their results, as confirmed in this laboratory, point to the general conclusion that even very low temperatures do not destroy the proteolytic ferments, although the activity may ultimately be impaired.

A barely significant change is shown in the activity of solutions of four crystalline enzymes frozen for 44 hours at -186°C . (Table 1). The preliminary nature of these experiments makes it impos-

TABLE 1
Stability of Solutions of Crystalline Enzymes Frozen in Liquid Air

Enzyme	Method of activity determination ¹	Activity after 44 hours' exposure ²
		pct.
Chymotrypsin.....	Formol titration	112 ± 8
	Soluble nitrogen	96 ± 8
	Milk clotting	80 ± 10
Pepsin.....	Formol titration	81 ± 8
	Milk clotting	60 ± 10
Trypsin.....	Formol titration	80 ± 3
Carboxypolypeptidase.....	Formol titration	85 ± 3

¹ See Table 2 for details of the *formol titration* and *milk-clotting* technic. The *soluble-nitrogen technic* was carried out as follows: To 6 c.c. of reaction mixture (3% casein, M/10 phosphate, pH 7.7) was added 2 c.c. of 1.6 M acetate buffer of appropriate pH so that the final pH was 4.3 ± 0.2 measured by the glass electrode. This pH region was previously shown to yield satisfactorily reproducible results when a uniform precipitate was obtained by shaking during and after addition of the acetate. The nitrogen content of the filtrate was determined by the micro-Kjeldahl method. ² The per cent activities are based on the activity of unexposed enzyme equal to 100. Initial activities, obtained by extrapolation to zero time, were employed in all cases reported in this paper.

sible to attach much significance to the differences, particularly with regard to the milk-clotting data. It is evident, however, that no great change in the activity of these enzymes is brought about by such exposure. Changes in the physical nature of biological material, dispersion of colloids, etc., in so far as they affect enzymic activity, have received considerable attention from Nord (1936).

EFFECT OF TEMPERATURE ON RATE OF TRYPTIC, CHYMOTRYPTIC, AND PEPTIC PROTEOLYSIS

The data show the relative rates of the hydrolysis of casein between 30 and -1°C . (86 and 30.2°F .) by crystalline trypsin, pepsin, and chymotrypsin (Table 2, a). As determined by the formol method, the values are not out of line with those reported in the older literature for mixed enzymes. It is possibly of greater interest, however, that within the limitations of the measurements, temperature coefficients of the three enzymes are alike. For chymotrypsin and pepsin

the time courses of proteolysis at 30° and —6°C. (supercooled liquid) are superimposable (Fig. 1), thus showing that the temperature coefficient is practically the same throughout the entire proteolysis.

It is evident from many facts that these proteinases do not all function in the same way. Chymotrypsin seems particularly adapted to the disintegration of unheated crystalline egg albumin, where trypsin is practically inactive. Trypsin, on the other hand, hydrolyzes casein more than twice as fast, and sturin 35 times as fast, as

TABLE 2
Activity of One Commercial and Four Crystalline Enzymes at Several Temperatures¹

(a) Relative initial rates of hydrolysis (rate at 30°C. = 100)				
Enzyme	Centigrade temperature			
	4°	—1°	—6.7°	—17.8°
Commercial trypsin.....	9 ± 1
Commercial trypsin ²	2	1
Pepsin.....	4 ± 1
Pepsin ²	3 ± 1	2 ± 0.5	<0.1
Chymotrypsin.....	9 ± 1
Chymotrypsin ²	1.4 ± 0.4	0.6 ± 0.2	<0.1
Chymotrypsin ^{2,3}	0.015
Trypsin.....	11 ± 2
Carboxypolypeptidase ³	8 ± 2
(b) Relative milk-clotting activity (rate at 30°C. = 100)				
Enzyme	Centigrade temperature			
	13°	—1°		
Pepsin.....	<0.2	<0.1		
Chymotrypsin.....	6 ± 1	<0.1		

¹ Methods: (a) The hydrolysis was followed by the formol titration as recommended by Northrop (1932) and by Northrop and Kunitz (1932c). The casein concentration was three per cent, except as noted above. The substrate for carboxypolypeptidase was chloracetyltyrosine. (b) Twenty per cent milk solution was prepared according to Kunitz (1935). Five-c.c. samples of this solution plus one c.c. of enzyme solution were placed in test tubes and the time required for curd formation was observed. The time required for curd formation or clotting at each temperature was determined for several enzyme concentrations to be sure that the activity (1/time) was proportional to the enzyme concentration. ² 1.5 per cent casein in 87 to 43 per cent glycerine by volume. ³ 30°C. experiments were not performed simultaneously with the low-temperature experiment in these cases.

does chymotrypsin; whereas chymotrypsin clots milk more than 100 times as efficiently as trypsin. Yet trypsin is at least 700 times as efficient as chymotrypsin in clotting blood, according to Kunitz and Northrop (1935). These variations are logically interpreted as due to an attack on different groups in the substrate by each enzyme. This conclusion also follows from the observation that rapid hydrolysis again sets in on the addition of one enzyme to a substrate already completely digested by another. Thus the total hydrolysis of casein

by trypsin and chymotrypsin together is about equal to the sum of the individual totals, Kunitz and Northrop (1935). A similar condition exists for pepsin and trypsin, Northrop and Kunitz (1932b).

Since the reactions proceed differently with each proteinase it was a bit unexpected not to find different temperature coefficients for each proteolysis. Proteolysis, however, undoubtedly consists of many chains of reactions, both branched and unbranched. Since the velocity measured by titration methods is really a summation of these various dependent and independent reactions, it is difficult to attach

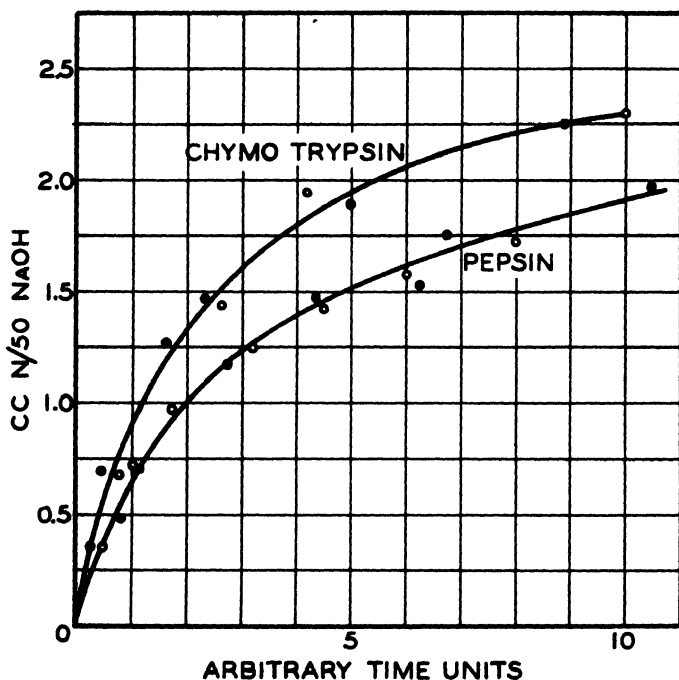


FIG. 1. Relative course of casein hydrolysis by crystalline pepsin and chymotrypsin at 30°C. (86°F.) and in supercooled liquid (formol titration).

The time scales have been adjusted so that the curves superimpose, i.e., the usual relation $(E)t = k$, where (E) = enzyme concentration, t = time, and k = a constant, has been assumed for any one temperature. The curves correspond approximately to one and 40 units of chymotrypsin at 30°C. (open circles O) and -6°C. (21.2°F.) (closed circles ●), and to one and 25 units of pepsin at 30°C. (open circles O) and -1°C. (30.2°F.) (closed circles ●) respectively.

any particular significance to the implied similarity of the "energies of activation" for these proteinases.

Extension of the temperature range to -18°C. (-0.4°F.) was made possible by determining proteolysis in the presence of 40 per cent glycerol, which is only mildly inhibitory. Comparison of the

activities in the glycerol-containing systems shows, as was the case in water, that both chymotrypsin and pepsin are affected to about the same extent by lowering the temperature of digestion (Table 2). At the same time it was evident that glycerol is much more inhibitory to all the proteinases at low temperatures than it is at high temperatures. This may be due to the increased viscosity of the glycerol system as the temperature becomes less, for, as appears later when the solid state is reached, proteolysis measured by titration becomes almost negligible; or the increased inhibition by glycerol may indicate the existence of a temperature coefficient for inhibition. In either case it shows that a "preservative," useless at ordinary temperatures, might still be quite efficient in cold storage.

The apparent temperature coefficients for milk clotting by the crystalline enzymes, pepsin, and chymotrypsin are shown to be much higher than for proteolysis (Table 2, b). Indeed, the milk-clotting activities of the two enzymes are affected so differently that pepsin is relatively much less efficient in clotting milk at 13°C. (55.4°F.) than is chymotrypsin.

PROTEOLYSIS IN THE SOLID STATE

Attempts to demonstrate hydrolysis in the solid state encountered serious technical difficulties. Casein (Table 1) and chymotrypsin were mixed at 0°C. (32°F.), immediately frozen in liquid air, and then placed in a thermostat at -6°C. (21.2°F.). After any desired time, formaldehyde was added and the contents of a tube rapidly thawed by shaking in a stream of hot water. Titration was then carried out in the usual way. Thus measured, the rate was found to be zero or at least not more than two per cent of that occurring in supercooled liquid at the same temperature. Another technique, based on the measurement of nonprecipitable nitrogen, however, showed a slow increase in soluble nitrogen (Fig. 1).² This method, which is more sensitive than the formol, may detect changes in the protein before sufficient carboxy groups have been liberated to be detected titrimetrically, according to Kunitz and Northrop (1935, p. 313). In an experiment at 30°C. (86°F.), when 20 per cent of the originally insoluble nitrogen had become soluble, an increase of about .3 c.c. of N/50 NaOH was observed. A titration of this order, alone barely significant, has been obtained frequently in solid-state experiments when 20 per cent of the insoluble nitrogen had become soluble. Therefore, it may

² The usual protein-nitrogen method, Northrop and Kunitz (1932c), using trichloroacetic acid, yields an activity comparable to that reported in Fig. 1.

be concluded that carboxy groups are liberated in the frozen state in about the same manner as in the liquid.

IMPORTANCE OF ENZYME ACTION IN THE COLD

Since the digestion of a protein is a series of reactions, it is not unreasonable to assume that some of the steps, most probably the first steps, are not adequately measured by our present methods. This is particularly true of the clotting reactions which seem to represent a very early stage in protein digestion. Any previous changes in the chemical condition of milk before it clots with pepsin have so far

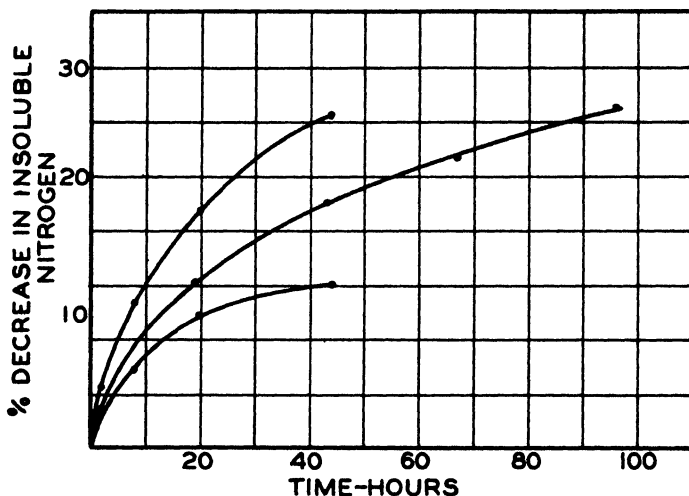


FIG. 2. Activity of chymotrypsin in the solid (frozen) state as followed by decrease in protein nitrogen.

The curves were obtained using 2.5, 1.5, and .5 mg. of magnesium sulfate dried chymotrypsin per six c.c. of three per cent casein at $-6^{\circ}\text{C}.$ ($21.2^{\circ}\text{F}.$).

escaped notice, yet such previous changes do occur and are of considerable importance in the final result.

After extended exposure to $-1^{\circ}\text{C}.$ ($30.2^{\circ}\text{F}.$) the clot forms rapidly in pepsin-milk solutions brought to $30^{\circ}\text{C}.$ ($86^{\circ}\text{F}.$) (Table 3). The observed clotting time of about one minute is scarcely time enough to warm up the milk tube from zero to $30^{\circ}\text{C}.$ The actual time of clotting has been reduced from 48 minutes to practically zero minutes by long-continued contact of enzyme and substrate in the cold. In the case of milk then, except for the physical manifestation of clotting, the action of pepsin at $-1^{\circ}\text{C}.$ differs from that at $30^{\circ}\text{C}.$ only in rate. Furthermore, the difference in rate is the same as that observed for proteolysis of casein measured by the formol titration (Table 2). The latter data supplied the temperature coefficient for the

calculations (Table 3). The situation with chymotrypsin may be somewhat different. We have been unable to obtain consistent data because the chymotrypsin-milk solutions exposed to $-1^{\circ}\text{C}.$ and then returned to $30^{\circ}\text{C}.$ are unusually sensitive to shaking. This property is also evidently the result of enzyme action but has not as yet been measured. These differences between pepsin and chymotrypsin in the clotting of milk serve to emphasize the individual characteristics of the enzymes even when acting on the same substrate to produce, at least grossly, the same result.

It is evident not only that changes in the milk do occur previous to the visible reaction of clotting but also that they occur at a temperature of $0^{\circ}\text{C}.$ ($32^{\circ}\text{F}.$).

TABLE 3
*Effect of Exposure of Pepsin-Milk Solutions to $-1^{\circ}\text{C}.$
on Time of Clotting at $30^{\circ}\text{C}.$*

Time at $-1^{\circ}\text{C}.$ ($30.2^{\circ}\text{F}.$) (to/60)	Clotting time when brought to $30^{\circ}\text{C}.$ ($86^{\circ}\text{F}.$)	
	Observed ¹	Calculated ²
hours	min.	min.
0	48	48
$6\frac{1}{2}$	33	$32\frac{1}{2} \pm 3$
$8\frac{1}{2}$	24	27 ± 5
17	$2\frac{1}{2}$	7 ± 3
$23\frac{1}{2}$	$1\frac{1}{2}$	< 0
41	1	< 0

¹ The time values of zero to two minutes are almost entirely due to the time required to warm to $30^{\circ}\text{C}.$ about one minute. No attempt was made to distinguish between the physical nature of the clots formed. A solution was said to be clotted when a curd or clot was definitely visible to the naked eye. ² These figures were calculated by assuming the temperature coefficient for hydrolysis (Table 2) so that t calculated = $48 - 0.04 t_0$.

This experiment appears to us to be of fundamental importance in that it bears on the fact that tissues frozen and later thawed disintegrate more rapidly than tissues which have not been frozen at all. This phenomenon has been customarily explained by the assumption that freezing and thawing disrupt the cells, thereby permitting better access of enzyme to substrate. Such mixing doubtless does occur in thawed tissues and must contribute to the acceleration of tissue autolysis that is observed on thawing. The explanation, however, has never been completely satisfactory, for it has also been observed that food is more apt to disintegrate after long storage than after short storage, although in both cases freezing and thawing were done in the same manner. The experiment indicates that an important factor in the rapid autolysis of thawed-out material is the extent to which enzyme action has already progressed during the frozen period. In the case of milk clotting with pepsin there is no real reason to assume that the

final step is enzymic at all. The facts are equally well explained on the assumption that the clotting *per se* is a purely physical phenomenon, for the observed clotting time is in fairly good agreement with what could be expected if the whole reaction took place at zero, except that the end products were unable to clot until the temperature rose.

Experiments in this laboratory with proteinases have thus shown that the measurable rate of protein hydrolysis, at least for three different proteolytic enzymes, decreases with the temperature to about the extent that might be expected of any chemical reaction until a point is reached where the system freezes. Below this point, visible proteolysis is slowed down much more and becomes in fact practically undetectable by the methods in use today.

This is not an improbable finding, for the change from liquid to solid must severely limit the sphere of activity of the enzyme by preventing diffusion and thus also preventing new contacts between enzyme and substrate. These results might be quite different if the substrate were a solid that occurred in comparatively large masses through which the enzyme could, so to speak, eat its way. Such a system would not be altogether independent of diffusion effects, for accumulation of the end products would still hamper the reaction. Nevertheless, it might be expected to show more activity in the frozen state. It is thus possible that insoluble protein fibers are comparatively more vulnerable below the freezing point than are the soluble proteins. This is a question with which our technique has unfortunately not been able to cope, but the well-known fact that hydrolysis of a fibrous protein goes faster along the fibers than it goes across them is of interest here.

There is, however, another common group of enzymes, the lipases, that act on large solid particles of their substrates. Although it is necessary, especially in working with solid fats like palmitin and stearin, to use fine emulsions, nevertheless the size of the emulsified fat particles is enormous compared with any reasonable conception of a lipase molecule. It therefore seemed worth while to investigate the possibility of lipase action in frozen systems. The results show that this does in fact occur with surprising rapidity.

LIPASE ACTIVITY AT LOW TEMPERATURES

The technique for demonstrating lipase action has not been very satisfactory in the past; for instance, the hydrolysis of tristearin, which is not by any means an abnormal or unreasonable example of a fat, can scarcely be demonstrated beyond the probable limits of error by any of the older methods. A new technique employed by Balls, Matlack, and Tucker (1937), however, in which tristearin is

dissolved in hot glycerine containing dried bile, then cooled, and diluted with buffer, permits this fat to be rapidly and completely split on the addition of a little lipase. This method of preparing fats for testing against lipase was thereafter followed in all cases, because it seemed that the most nearly complete picture of the action of the enzyme could thus be obtained.

The behavior of the series of saturated fats toward pancreas lipase was studied. The following data are taken from the work of Matlack, Tucker, and Balls:

Under otherwise comparable conditions the effect of the temperatures on rate of digestion was very marked, for instance, speaking approximately in order to make a clear generalization, tristearin digests rapidly at 40°C. (104°F.), but only slowly at 30°C. (86°F.); tripalmitin, trimyristin, and trilaurin all digest rapidly at 30°C., but only slowly at 20°C. (68°F.). From tricaprylin to triacetin, however, the picture is different. Tricaprylin, tricaproin, trivalerin, tributerin, tripropinin, and triacetin all digest at a speed almost independent of the temperature between 20 and 40°C. The fats to go fastest at any given temperature are those with fatty acid chains about six to eight carbon atoms long, and triheptylin, with an odd number of carbon atoms, does as well as any. The data also show that olive oil (mostly triolein) behaves at different temperatures not like a C_{18} fat, but like a C_8 or C_6 fat.

If the behavior of the systems that react at 20°C. is investigated at 0°C. (32°F.) (where the emulsions are still liquid), it is found that hydrolysis goes on much more slowly. A day at 0°C. is about equivalent in effect to an hour at 20 or 40°C. When the temperature of such lipase systems is further lowered to about -12°C. (10.4°F.), they freeze, but the difference in initial velocity is slight between systems that are liquid, pasty, or quite solid. The differences in state were obtained by changing the concentration of the glycerine in the system slightly.

Even at -30°C. (-22°F.) splitting takes place with a velocity appreciably greater than any probable experimental error. The actual extent of splitting would probably be much greater still if the liberated fatty acids could be neutralized. This is done in the regular procedure with liquid systems but it is impossible with a solid.

The results with olive oil, expressed as initial velocities, are shown (Table 4).

Lipase action, therefore, proceeds at low temperatures and in the frozen state. The lipase is apparently more active than the proteinases and can be demonstrated with certainty in the course of a few days.

Only the lower saturated fats and the unsaturated fats are measurably affected at such temperatures.

The practical implication of these findings is evidently that lower fats and unsaturated fats are not well protected from enzyme action by lowering the temperature, while the higher saturated fats are. Theoretically butter, for example, should not keep very well in cold storage.

The difference in behavior between the higher fats and the lower fats with lipase at different temperatures is so very marked that one might almost say the *specificity* of lipase varies with the temperature.

TABLE 4
Time Estimated for Five Per Cent Splitting of Olive Oil
(Pancreas lipase)

Temperature	Time	Notes
°C.	days	
+40	0.0008	Continuously buffered —not frozen.
+30	0.0016	
0	0.06	
— 6.7	0.25	
—12	0.5	
—12	0.8	Not buffered —not frozen.
—12	0.9	Not buffered —frozen.
—30	7.2	

CONCLUSIONS

Results have been interpreted here to mean that enzyme action at low temperatures not only takes place but is an important factor in problems of food preservation. In general the data presented show that in some cases, notably with lipase, the velocity is, all things considered, quite surprising. In other cases the extent of enzyme action is so slight that it cannot be readily or certainly detected. It is even then of importance, however, because the first phase of an enzymic attack on the substrate may have been completed, and when the material is brought to ordinary temperature again, the evident action of the ferment occurs all the more quickly because of the partial reaction in the cold.

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MODERN PRACTICE IN FISH PRESERVATION BY COLD

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In the whole of eastern Canada, especially in Quebec, there was, for more than 200 years, an extensive production of salted, dried codfish for export. Ninety per cent of the total production of its fishing industry was marketed in this form, Italy being the principal market. Changes in economic policy in that country necessitated a conversion of the industry to production of fresh and frozen fish for the domestic market in a very short time. No better field, however, could be found to demonstrate clearly the help that research workers can bring to the engineer and what solid foundations science can give to the evolution of industry.

The situation in the fresh- and frozen-fish industry was at one time not very definite nor, in some ways, very interesting. A demand for fresh sea fish had been created all over the country and fresh, unfrozen fish was sold outside of what could be called the areas of safe marketing. The surveys in markets distant from the seashore showed fish still edible, yet many times it would have been quite an undeserved compliment to call it fresh. In the frozen-fish field the situation was not any more encouraging. With depression prices in force, producers had to cut their cost and many reverted to the old still-air freezer.

Our natural markets in Canada were already supplied through other channels. The yearly per capita consumption of fish in Canada is quite low, 20 pounds against more than 50 in many European countries. Publicity is a good medium for increasing consumption but scientific production is still better; a product of quality writes, by its intrinsic value, its own advertisement.

FRESH FISH

In the fresh-fish field, science had already established:

- a. That though decomposition of the flesh of fish is due to two agents, enzymes and bacteria, bacteria are the principal agents; and if they can be checked, preservation of fish in a fresh state can be lengthened.
- b. That the muscle of fish is practically sterile. Fellers, working on raw salmon spoilage, made 18 tests and declares: "In every one, the flesh was sterile." Van Driest, working on haddock,

has reported that in some instances bacteria were present in muscular tissue in considerable numbers but the general belief is that there are ordinarily not enough to cause any harm.

- c. That the sources of spoilage are limited principally to blood and slime; so the first parts of the fish to be attacked by decomposition are the skin, gills, and intestinal tract.

On the other hand, the limitations of a moderate cold (above the freezing point) are no less well known. Down to the freezing point, cold cannot stop the action of bacteria; it can retard them, but they spoil the fish after a time.

If fish were to be kept in a fresh state, another way had to be found to hold bacteria in check. Industry tried to forge ahead of science at this point, and some audacious packers used, to our knowledge, anhydrous sodium sulphite or salicylic acid. Let us say that such practice is fortunately a thing of the past. Science has since found mild sterilizing agents, harmless to humans, and prescribed rules for their use. The two best ones are chlorine and hydrogen peroxide. They kill bacteria and then disappear, leaving no traces. Chlorine is used at a concentration only twice as strong as for the purification of drinking water; and hydrogen peroxide, though rather slow in its bactericidal action, has a desirable bleaching action. Both are volatile, however, and hard to keep in constant solution. They are used in large plants where an effective and constant control is possible.

For smaller plants with less skilled labor a simpler bactericide is needed. The ideal one would have to answer the following specifications: be perfectly harmless to humans; rapid-acting so as not to delay shop routine; easily dissolvable in water, giving an homogeneous solution; simple of handling, so as to be entrusted to average skilled hands; capable not only of killing bacteria but of improving the flavor or the appearance of fish.

So far science has given us many preservatives and they come near to this ideal. They are used quite extensively. One of them, the English "Keeps," is a mixture of boracic acid, borate of sodium, and sodium chloride in the proportion of 80-15-5. None of these preservatives, however, is the ideal itself, and proof that such ideal has not been discovered yet is contained in the following advertisement read in "La Revue du Froid" of Paris, January 1937 issue:

"The Cold Storage Society of Bremen, in Germany, has just offered a reward of 25,000 marks to the Scientist who will find a chemical substance which, added to ice, will preserve fish three full weeks at least, in prime condition. Such chemical, however, to be harmless in human consumption, not to lower the freezing point of water and not to attack metal."

The offer was good up to the first of July. Up to June, no hint was given in "La Revue du Froid" that the offer had been redeemed. So we are still waiting and, though our preservatives have given good satisfaction, as soon as a better one is discovered, it will be used.

There are two ways of applying preservatives to fish. The more popular is to turn them into an homogeneous solution in which they are thoroughly washed by a few minutes' dipping. The concentration actually used for such purposes is ten pounds of powdered preservative to ten gallons of clear, pure water. This is a quite concentrated solution but the fish is simply dipped in and when it reaches the consumer's table, all the preservative has been drained or washed off. The other way is to incorporate the sterilizing agents in the ice used for packing fish. A solution of dilute boracic acid is often used and is said to keep fish in good condition for two weeks, or even longer in the case of halibut.

Fish has a point in common with milk. It needs a good cooling as soon as caught, and the faster the cold penetrates the longer the product will stay good and fresh. There are many ways to cool fresh fish down to the vicinity of the freezing point. It may be done with an ordinary air cooler. This method is safe but it is the slowest way, since still air extracts heat at a rate five times slower than a liquid solution; moreover, it brings a certain amount of desiccation on the outer surface of the fish itself.

This conductivity of liquid solution is used in the packing of fish in crushed ice. In Quebec we have something better than ice; we have snow. In fact, snow is sterile ice prepared by nature itself and shed on earth at a time of the year when there are few bacteria in the air. Stored in convenient ice-houses at very cold temperatures, snow keeps well. Being very finely divided it adheres to fish more evenly than ice, and in melting it extracts heat more rapidly. In addition, it has an insulating property. When the temperature of the flesh of fish has reached an even $0^{\circ}\text{C}.$ ($32^{\circ}\text{F}.$), snow does not melt any longer. It becomes an insulating material and keeps the fish cold without freezing it. When packed in strong, tight boxes, fish buried in snow will keep longer than in ice and it can be kept from freezing as well as from re-heating. In fact, in a cold month of December we have buried fresh steak cod in snow, and after 15 days with an outside temperature of often $-17.8^{\circ}\text{C}.$ ($0^{\circ}\text{F}.$) we found the fish a bit *stiff* but yet *unfrozen*. This can be easily understood. At $32^{\circ}\text{F}.$ snow stops melting; fish does not start freezing before reaching -2.2 or $-1.7^{\circ}\text{C}.$ (28 or $29^{\circ}\text{F}.$). The minute particles of air enclosed in the snow shut out the outside cold and the fish is kept but cannot freeze. This two-fold property of snow has made it more popular than ice.

An indirect way of helping to keep fish for a longer period in a fresh state has been its dressing into steaks and fillets. In this way the heads, gills, blood, and flanks are discarded; they are parts in which putrefaction works fast. At the same time the industry has complied with the wish of housekeepers who want fish ready for cooking.

FROZEN FISH

Let us see now what pure science had to offer to help in the production of a frozen fish of quality. First, it has found out that real fresh fish, in state of rigor mortis, has no free water in the cells of its muscle but only a gelatinous solution; and that during the following stages of digestion by bacteria this jelly-like substance is decomposed into simpler nitrogenous products, mineral salts, and free water. So, if a fish is frozen hard in the state of rigor mortis and well kept in storage, it will not thaw out any older from a decomposition standpoint than it was at the time of freezing.

Science teaches too that if fish are "frozen rapidly, the gelatinous contents of the cells composing the muscle solidify as a mass of frozen jelly, whereas if freezing takes place slowly there is a tendency for the water content of the cells to separate from the jelly-like mass and rupture the delicate cell walls, so that when the fish is defrosted the juices are free to escape. Secondly, it has been found that the maximum damage to the proteins of the fish muscle during freezing and thawing occurs while the muscle is in the temperature zone 32°F. to 23°F. and that the damage done is roughly proportional to the time the muscle is in that zone of temperature."

Another established scientific principle is that air is a poor conductor of heat but a good convector when kept in constant circulation, and that a liquid solution is a very good conductor and acts by convection too when kept in circulation.

From these premises, one might conclude in favor of freezing foods in liquid solutions and by the use of very low temperatures. So did we at the start. Brine-freezing patents flourished everywhere, and even on our small and remote Gaspé Coast we had four cold-storage plants equipped with Linde-Taylors systems, direct contact, and one with a Petersen indirect contact. So far as temperatures were concerned, it was the time when the Birds Eye Laboratories operated their belt system at temperatures as low as -55°F., and we operated our Petersen plant on the coast at -35°F.

All have come back from that first-hand enthusiasm. In modern technology, in eastern Canada, still air will never come back, but circulating air or "Coldblast" has taken a rather large expansion.

Compared with still air, it cuts the freezing time in half; an experiment run last year by the writer illustrates this fact in a very clear way. Two large halibuts were put in freezers, one with a circulating fan, the other in still air, and frozen. Here is a comparison of results:

	With circulating air	With still air	Difference in flavor
	<i>hours</i>	<i>hours</i>	<i>hours</i>
Cooling zone (down to 32°F.).....	6	14	8
Critical zone (32°F. to 22°F.).....	19	32	13
Storage zone (22°F. to 13°F.).....	8	15	7
Total.....	33	61	28

Frozen fish from Canada, especially frozen salmon, has found its way to English markets and received many favorable comments for its good texture and quality, even from discriminating fish buyers on the Billingsgate Market. For round and specially large fishes "Coldblast" has given satisfaction; moreover, it is simple and easy of operation. The fish is loaded on pans or hung on racks and placed in corridors where a cold, damp draft is delivered at the rate of 1,000 cubic feet per minute. It was said at first that this system dried out the fish to a considerable extent. To lessen this drying, two ways were devised: dipping the fish in water before exposure to the cold draft or stopping the fan for the first hour or two of freezing, until a small outside layer of fish was frozen stiff and checked any further evaporation.

"Coldblast" or circulating air cannot, however, take large fishes through the critical zone of crystallization fast enough to enter them in the class of "quick frozen" fishes. Nor can this be accomplished for the large fishes with a brine solution. The efficiency of a brine solution decreases with the thickness of the fish, and from three inches up, no fish could be called "quick frozen," whatever system of congelation was used. So another problem presented itself. The quality of a "quick frozen" product was well known and asked for by consumers. To comply with this wish, large fishes were cut into small portions, packaged in cartons, and frozen in the containers through "frigo" plates or under a spray of brine. The packaged fish trade was thus brought into being.

To freeze fish quickly is an important thing. No less important in modern technology is the care of some fish after it is frozen. Round fishes have to be glazed. Water has been used and is still much in use, but it evaporates and allows cracking to take place; so the fish have to be watched closely and reglazed quite often during the storage

period. In the last two years an antiseptic and non-cracking glaze has been discovered and put on the market. It is a solution of some chemicals in tap water in definite proportions, boric acid, disodium hydrogen phosphate, anhydrous or hydrated sodium sulphate, and calcium lactate being used. This particular glaze, however, is rather whitish and opaque. It was prepared for halibut and did not mar the appearance of this particular fish. On salmon for export to England, the result was poor and our consignees asked us not to use it.

Sometimes scientific discoveries are made in a peculiar or quite novel way. When we wanted to stick to brine freezing at whatever cost, we tried to find a colloidal solution of organic nature that would be salt-proof so that fish dipped in it could be exposed later to the action of brine with no salt penetration. The mixture was prepared and tried on smelts but the results were just the reverse of our expectations. This particular salt-proof solution absorbed salt and revealed itself as a salt-loving solution. In fact the coated smelts averaged two per cent more salt than the uncoated, but this antisalt solution was not salted away. The coating was quite transparent and enhanced the bright colors of the fish instead of dulling them as does ordinary ice glazing. We are working now on the commercial production of this particular substance and intend to use it for glazing our fish.

Next to a good glaze, fish requires a low and equable temperature for storage in order to prevent crystallization in cells and subsequent softening of the flesh. It is universally complied with in modern practice. The excess of cold production in the ammonia machines is diverted to rooms and very seldom does the temperature go above 0°F.

A safe control of bacteria in fresh fish, a sharp cooling right after fish is caught, and the delimitations of reasonable marketing areas are the main gifts of science to the safe handling of fish in a fresh state. A specified time for freezing, a quick processing, and safe storage conditions are the same gifts to frozen-fish technology. They have enabled us to put on the market a fresh fish of quality within reasonable areas around fishing centers; they have also enabled us to put a high-quality frozen fish on the market and to insure the safe delivery of sea fishes, round or in packages, far inland and even across continents and oceans.

The safe conversion of our dry, salted fish industry into a fresh- and frozen-fish business is not terminated, but, with these contributions of science, to practice it is well under way.

COLOR OF MEAT

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It is unfortunate that color of meat should be due to the presence of such a complex and relatively unstable compound as haemoglobin. Fixation of color during curing is the traditional method of overcoming this difficulty.

The undesirable properties of haemoglobin from the point of view of preserving color are counterbalanced to a large extent by another factor, the muscular tissue's uptake of oxygen.

OXYGEN UPTAKE OF MUSCLE

Muscle, after rigor, retains indefinitely a residue of its respiratory activity. When exposed to air, therefore, a steady state is reached where the depth, d , to which oxygen penetrates, is determined by the relative rates of its diffusion and uptake. The relation is given by the equation

$$d = \sqrt{\frac{2c_0D}{A}}$$

where c_0 is the pressure of oxygen at the surface of the tissue, and D and A are respectively the coefficients of its diffusion through the tissue and consumption. The value of A for the muscle of fresh beef and pork as reported by Brooks (1929, 1936) is roughly 10^{-4} c.c. per gram per minute at $0^\circ\text{C}.$ ($32^\circ\text{F}.$), the uptake decreasing slowly with time. For bacon A is smaller, of the order of 3×10^{-6} ; the presence of relatively high concentrations of sodium chloride decreases the uptake of oxygen.

The corresponding values of d are roughly .2 cm. for beef and pork and .4 cm. for bacon at $0^\circ\text{C}.$ The depth to which oxygen penetrates decreases with increasing temperature. It also increases slowly with time, but rarely exceeds one centimeter, even after very long periods of storage.

In lean meat and bacon exposed to air, therefore, dissolved oxygen is present only in a superficial layer a few millimeters thick; below this layer the tissue is completely devoid of oxygen. Both haemoglobin and nitrosohaemoglobin (the pigment responsible for the color of bacon) change to the brown pigment, methaemoglobin, in the presence of oxygen, but both are stable in its absence. Discoloration is confined,

therefore, to a thin superficial layer. The absence of oxygen in the bulk of the tissue also determines the path of reaction between nitrite and haemoglobin, the basis of fixation of color.

FORMATION OF METHAEMOGLOBIN IN MEAT

The color of lean meat is brownish when roughly 60 per cent of the haemoglobin present in the superficial layer has been oxidized to methaemoglobin, Brooks (1929, 1933). Haemoglobin is present in the fat of beef, but the rate of oxidation, except in local areas, is slower than in the lean.

Oxidation is very slow at low temperatures; at $-10^{\circ}\text{C}.$ ($14^{\circ}\text{F}.$) more than 16 weeks are required for any visible discoloration to occur. The formation of methaemoglobin in frozen meat is much less important (unless the time of storage is unduly long) than loss of color owing to excessive drying. Here, crystals of ice in the superficial layer evaporate, and the small bubbles of air left behind scatter the incident light. The same cause is responsible for the discoloration of frozen offal.

With quarters of chilled beef—shipped at $-1.4^{\circ}\text{C}.$ ($29.5^{\circ}\text{F}.$)—there is usually little or no discoloration owing to the formation of methaemoglobin up to about 40 to 45 days from killing. Some discoloration, chiefly of local areas, is found with longer periods, the amount depending on quality of the meat. In some cases a satisfactory color has been maintained for 60 days.

These times for chilled beef refer to commercial conditions of storage. The onset of discoloration is closely connected with the factors governing loss of water from the meat. At high humidities, for example, 99 per cent R. H. at $0^{\circ}\text{C}.$, exposed muscle is discolored by methaemoglobin in 20 to 30 days; on the other hand, excessive drying gives an unpleasantly dark appearance, owing to optical changes in the tissue (no pockets of air are left behind). There is, therefore, a desirable loss of weight—a moderate increase in the depth of color owing to drying masks the change in hue caused by the formation of haemoglobin and, in addition, haemoglobin is apparently oxidized more slowly in the partly dried tissue.

Other factors influence the rate of oxidation of haemoglobin to methaemoglobin, for example, pH and the pressure of oxygen. The rate increases rapidly with decreasing pH, as shown by Brooks (1930, 1931). The very rapid discoloration of meat which has been in contact with brine is mainly caused by a change in pH and, to a smaller extent, a "salt effect." The large increase in ionic strength because of the penetration of sodium chloride into the superficial layers of tissue lowers the pH of the buffers present. Given a sufficiently high con-

centration of sodium chloride, the rate of oxidation of haemoglobin may be increased four to five times.

The influence of the pressure of oxygen on rate of oxidation is interesting from the point of view of mechanism of the reaction. The rate increases with decreasing pressure and reaches a maximum at low pressure (of the order of four mm. O_2 at $0^\circ C.$), Brooks (1929, 1935). The quantitative relation is important in fixing an upper limit to the concentration of carbon dioxide which can be used over a given period in the gas storage of meat.

FIXATION OF COLOR

As already stated, absence of oxygen in the interior of muscular tissue of bacon insures the stability of the pigment, nitrosohaemoglobin, Brooks (1936). It also plays an important part in the process of fixing the color. The action of nitrite on haemoglobin in the presence of oxygen is not yet clearly understood; whatever the products, nitrosohaemoglobin is not the chief one, and the color of the resulting mixture is usually brown and, in some cases, green.

In the absence of oxygen, nitrite reacts with haemoglobin in aqueous solution to give one molecule of nitrosohaemoglobin and one of methaemoglobin, Brooks (1937). Again the color of the mixture cannot be considered good. If, however, a reducing agent, e.g., sodium hydrosulphite, is also present in the solution, nitrosohaemoglobin is the sole product. These latter conditions are found in the interior of muscular tissue during curing; in the absence of oxygen, tissue maintains a low value of the oxidation-reduction potential (E_h is of the order of -2 volts) and haemoglobin is changed completely to nitrosohaemoglobin.

The reaction of nitrite with haemoglobin is relatively slow over the pH-range 7.8 to 7.2, but the rate increases with decreasing pH and is rapid over the range 6.6 to 5.2. The reaction is very rapid at the concentrations of salt and pH found in muscle during curing; the time required for the fixation of color must be attributed to the time necessary for adequate diffusion of nitrite into the tissue rather than to any property of the reaction.

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IMPORTANCE OF THE UNIT OPERATION CONCEPT IN FOOD ENGINEERING

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It has been my confident belief that some day an occasion would arise when men from the various branches of food manufacture would assemble to discuss common problems of production and quality control in broad terms of the common denominators that run through all food-manufacturing operations.

What are these common denominators? What logical basis of mutual interest exists that will convince the baker and the ice cream maker that they can sit down together and talk over a common technical problem? How can the superintendent of the dry-sausage department of a meat-packing house be convinced that he has an operating problem identical with that of any macaroni manufacturer? In what language will the dairyman and meat packer discuss technical and operating matters identical with certain every-day problems that confront the master brewer? These are not hypothetical or academic questions. Without much effort, the list of different branches of food industries which have many identical problems could be extended to huge proportions.

If we consider *what* is being done in food factories without dwelling too much on what raw material is being treated, we soon realize that often the same thing is being done in each plant, using different materials. We find that cleaning, washing, scalding, pumping, concentrating, mixing, sterilizing, disintegrating, sifting, freezing, grinding, and the like are processes common to many plants; or that hydrolyzing, baking, coagulating, percolating, refining, reducing, oxidizing, and fermenting are usual procedures in many other factories.

What common denominators have we in the business of manufacturing foods other than the unit actions, or unit methods, unit operations, and unit processes which in their proper sequence constitute the process by which the manufactured food is produced?

Now, instead of 33 separate industries we have only one—the food industry. Yet how “compartmentalized” is our knowledge and our experience in food-manufacturing operations! Something comparable to a Chinese wall insulates the thinking of most men in one branch of the food industry against the other branches. On every side we hear the complaint, “But my business is different.” Men in the food

industries have been emphasizing the dissimilarities of their businesses for countless years, utterly ignoring the fundamental similarities.

The basic reason for my protest is this: There are examples where the man in a food business is facing a problem of operation that all too frequently has been completely solved by another branch of the food industry—a branch which he knows next to nothing about and from which he is certain he can learn nothing because his raw materials and his finished products are different from the other fellow's. Often the solution to his problem could be his for the asking, but in all too many cases he cannot formulate the question he should ask, nor can the other fellow formulate the answer in a language that both can understand.

At this point, I wish to state parenthetically that the intellectual Great Wall that exists in our minds when we consider technical industrial operating problems is not peculiar to the food business alone. It exists on all sides and its very existence is something of an indictment of our educational system.

I recall a conversation in 1931 with Dr. Walter Polakov, one of the greatest engineers in this country in the field of power-plant design and operation as well as construction. We were talking about this very point—the resistance of the human mind to an idea that arises from another source outside our daily experience. One of the biggest problems he had to cope with was that of refuting the recurring objection, "My business is different."

Within recent months I have had a parallel experience. In June of this year I was standing in the sausage-drying room of a meat packer out in the Missouri River valley. My escort was in charge of all cured-meat manufacture for that company. He told me he was certain that great improvements were ahead in the methods of curing and drying dry sausage, that their engineering department and their laboratory were studying the problem, and that he rather suspected air conditioning was to be the solution.

"Solution of what?" I asked him.

"Case hardening," he replied. "It is easy to take out a requisite amount of water up to a certain point, but at that point we run into the problem of a hard, dry layer forming on the outside of the sausage, something like the rind on a cheese. That is objectionable. Furthermore, if the sausage case hardens, we find it nearly impossible to get out the rest of the moisture which must be removed. Sometimes considerable deterioration occurs on the inside before we can get it dry enough if we permit the case-hardening. We are doing a great deal

of research on it right now and I think we shall have it solved before long."

To his remarks I replied, "Your drying problem appears to be exactly the same as the job of drying macaroni, spaghetti, vermicelli, and noodles. We published a story on drying about four or five years ago that you ought to have read."

"You did? I must have missed it."

"As I recall it, the story bore a title that said something about dispelling the mystery of macaroni drying."

"Oh, I saw that, but that was about macaroni. I want something about *sausage* drying."

The foregoing incident illustrates the intelligent man who has lived so long in a single industry that he habitually thinks in terms restricted to it. In effect he has developed a characteristic which we sometimes see in college students as well as older people—a mental fixation which prevents them from solving a mathematical equation for an unknown unless that unknown is expressed by the letter x .

Quite in contrast to this episode is the example of another man of great intellectual curiosity and wide thinking and reading. He is production manager of a large ice cream company, one of the most successful in the country. His company had recently installed the tunnel system of hardening ice cream. This involved an insulated tunnel containing a conveyor which carries the ice cream in packages through a blast of refrigerated air. His unit, a very large one with a conveyor about 12 feet wide, had not been performing satisfactorily after it was installed. The ice cream had not been hardening uniformly. Investigation had revealed that the air movement was not uniform throughout the tunnel, and as a result the temperatures were not uniform when the plant was operating at capacity.

This man recognized his problem in terms of basic principles. In his own mind, it was a problem of temperature control—not merely a problem of continuous hardening of ice cream. While studying the problem, he read about methods of attaining good temperature control in large bake ovens. Such ovens may be from 80 to 100 feet long and from 10 to 12 feet wide. The fact that temperature in the oven was in the vicinity of $204.4^{\circ}\text{C.}(400^{\circ}\text{F.})$, whereas he was interested only in temperatures of $-26.1^{\circ}\text{C.}(-15^{\circ}\text{F.})$, did not confuse his thinking in the least. In each case he recognized an insulated conveyor tunnel, an air-blast, a mechanical means of changing the temperature of the air, and a system of controlling the temperature. This chap had never seen the inside of a big bakery, but his curiosity was not satisfied with reading, he had to see with his own eyes. Accordingly, he called up the bakeries in his city, soon he found one which had a big traveler

(traveling plate oven), and he asked permission to call and observe the methods of temperature control. His request was promptly rewarded with an invitation to "come right over." In that bakery he found a ready-made solution to his problem.

The same production manager has profitably utilized methods of other industries on several other problems related to the ice cream business, and my belief is that a great proportion of the material success of his company is attributable to his methods of thinking. For example, he puts up cold-packed strawberries for strawberry ice cream and, of course, must have the berries washed. The berries which he prefers, on account of their superior flavor, are grown in a sandy part of the country and present a washing problem of no mean order, for every particle of embedded sand must be removed. The method of washing which he uses is an adaptation of the method of washing sugar beets which he observed while traveling in the West.

As long as food manufacture remains only an art it will remain heterogeneous; it will not become homogeneous. In most branches of food manufacturing today there are countless centuries of prior art. The knowledge of *how* to produce most foods goes back beyond recorded history. Centuries of trial and error have developed an art—a "know-how"—that is still in advance of the science—the "know-why."

I suggest that we men in the food industries can learn much from our wives by a careful study of their mental approach to the daily task of cooking meals. I have yet to see the housewife who fails to understand that she is dealing with methods of manipulation irrespective of the particular raw materials with which she is working. If she is whipping, she does not need to buy one kind of machine from a dairy equipment company to whip cream, another one from a poultry raiser's equipment company to whip eggs, or still another from a confectionery equipment company to whip icing for a cake. Also, she knows the principle of baking, whether it be meat loaf one day, a cake on another, bread on a third day, or beans on a fourth. To her it is still baking.

The farm kitchen that is properly equipped for ordinary household use has every item of equipment needed to prepare almost any known dish. To be sure, the raw materials may be lacking—the housewife may be "fresh out" of bird's nests for a particular kind of Chinese soup; she may not have on hand clams for clam chowder; she may lack sharp-cured Parmesan cheese for a dish of spaghetti. She has the equipment, however, to utilize all these raw materials and the knowledge of how to manipulate them. She has been trained in methods.

The point for emphasis is that an intelligent housewife does not confuse a method with a food or culinary dish. At the other extreme men in industry often have so specialized on the production of a single item that to dissociate the methods employed from the food itself requires a conscious effort of considerable magnitude. Just recall the sausage superintendent previously mentioned—the man who was interested only in sausage drying, not in drying as a method.

Yet, despite the very obvious educational value of cooking experience as a training in methodology, I know of only one food technologist who has ever been a chef. I can testify that he is a splendid cook, an excellent technologist, and a very able engineer. Of course, we all know that the factory can do many things far better than they can be done in the household kitchen, but the prototype of the food engineer, in the qualitative sense, is personified by the cook.

Let us turn from the simple illustrations of thinking, or failing to think, in terms of principles to consideration of the principles themselves. What better term is there for these methods—these building blocks of the structure of technical knowledge of manufacturing—than the terms “unit operations” and “unit processes”? So far as I can ascertain, the concept of the unit operation first made its appearance in the Massachusetts Institute of Technology. When three members of the Chemical Engineering faculty of M.I.T., Professors Walker, Lewis, and McAdams, published a book on chemical engineering principles in which they treated chemical engineering for the first time as a group of unit operations, they changed the whole course of the profession. Rapid progress in chemical engineering dates from 1916-1918, when their basic thinking was made available to others than their students. They recognized that, regardless of the substance or substances being processed in the chemical industries, all of the manufacturing operations simmered down to a few fundamentals which they termed “unit operations.” They thought of everything in terms of washing, grinding, roasting, sifting, distilling, crystallizing, and the like. Chemical engineers who have graduated from their respective institutions since the appearance of the unit-operation concept in chemical engineering are better trained, more adaptable, more likely to solve today’s problems with quickness and dispatch than those who have never been trained to think of chemical engineering in terms of unit operations or unit processes.

In the field of food manufacture today there exists something of a counterpart of the conditions of chemical engineering as it was about 25 to 30 years ago. In that day only the chemistry of cement manufacture, sugar refining, gas manufacture, paper making, glue manufacture, and the like were studied. In each case emphasis seemed

to be on the chemistry of the raw material and the finished product with no thorough consideration of methods employed in achieving every step of production. Of course, there was no such thing as a quantitative technology of manufacturing methods; in fact, there was hardly existent even a qualitative technology.

In most larger fields of food-manufacturing industries there exists today schools where the art of production in a given field is taught together with a modest amount of explanatory science. Many are merely trade schools. There are schools of baking, brewing, canning, dairy manufacture, flour milling, meat packing, and sugar refining. Perhaps there are others. There is hardly a state college in the land that does not take pride in its dairy department. The individual can learn a great deal of the art of manufacturing in these schools or he can learn it in the hard school of experience, but the training of the student is usually that of a technician instead of a food technologist.

At present, the literature of food engineering is pitifully meager, despite the prototype which exists in the literature of chemical engineering, especially in the quantitative studies of the unit operations of chemical engineering. My own appraisal of the situation is that we have only a fragmentary literature of food engineering which, at best, is purely descriptive or qualitative.

The time is nearly here when we may expect the beginning of realization of the needs for a widespread study of unit operations, first on a qualitative or descriptive basis and later on a quantitative basis. How many years must elapse between the wholesale acceptance of qualitative food engineering and quantitative food engineering is purely a matter of conjecture. That both of them will come, in the course of time, I have no doubt. Competition in the industrial world will compel it. Stockholders of food companies are growing impatient for profits. On every side are evidences of efforts of men in the various branches of food manufacture to reduce costs, improve manufacturing methods, and institute more reliable forms of quality control. In the field of food manufacture, save for a very few notable exceptions, we are still in virgin territory.

It is the natural course of events in the progress of knowledge that one must first be able to recognize the thing he is measuring before he is able to measure it; hence, it is to be expected that descriptive technology must precede quantitative technology. Descriptive technology is not enough; there will be no profession known as food engineering until the quantitative approach is gained.

Technical knowledge in the various branches of the food industries is at very different levels. In some branches, the business is pretty

much a trade; in others, its technical knowledge is moderately advanced. In still others, such as sugar refining or the starch-derivatives industry, it is very highly advanced. I want to call to your attention, however, the fact that the greatest advances exist in the technology of production of foods where there are no obscure flavors or flavor constituents to be carefully retained because they render the food valuable. It appears also that where flavors are subtle, arising from minute amounts of unknown substances, the business of manufacturing foods partakes more of the art than of the technical science. Consider wine and coffee manufacture at one end and sugar manufacture at the other.

All of this has a marked bearing on the market for men who are food engineers. It is almost inconceivable that anyone today could operate a petroleum refinery or a pulp and paper plant without the aid of chemical engineering and still remain in business. On the other hand, the food business of today seems to get along fairly well without the food engineer, and all too often without even a chemist. In many respects the food engineer of today is about where the chemical engineer found himself many years ago—neither chemist nor engineer. Most manufacturers in that day had not the slightest idea what a chemical engineer was nor what they should do with him. The neophyte had only the sketchiest sort of notion about where he would fit into the industrial scheme of things. He had his youthful dreams of being superintendent or production manager or even president of the company—dreams based on the knowledge that others who had taken the same course of training had, by some mysterious process, achieved similar advanced positions.

In the chemical-process industries today, most men in the management function realize what chemical engineering is, what sort of jobs the chemical engineer can do best, as distinguished from the work of the research men or the analytical chemist. Chemical engineering, as a profession, could not exist in its present well-defined manner without the concurrent recognition by industrial management of its proper use.

Both chemical engineering and food engineering are closely related, involving many of the same industrial-scale unit operations and unit processes, often employing certain techniques that are specific for the type of problems involved.

What are these unit operations and unit processes to which I have referred so frequently? I have tried to show very briefly their place in the scheme of things, and now it is time to define them. My business associates and I have conferred frequently on the definition of

each, and our definitions were published in February, 1936 (*Food Industries*, p. 19, Feb. 1936). In spite of being faulty, they have not been challenged up to now, largely, one suspects, because nobody else has analyzed them carefully. Since then, we have redefined unit operation in the following words: "A unit operation in food engineering is the method by which an intentional or controllable change of form or place of food material or ingredient is effected." The unit process was defined in the following manner: "A unit process in food engineering is an intentional or controllable change in composition of a food material or ingredient."

Considering only unit operations, if one tries to think of every operation carried out in food manufacture, writes each one on a separate card, and then groups together those which are related or involve the same type of method, he will find very few classes compared with the number of names of operations. We found about 107 different names that we could think of, but these fall into only 15 groups. The unit operations in food engineering group themselves naturally into 15 different classifications. The subsidiary names in some groups are rather large. Up to now we have been able to distinguish only the following groups:

1. Cleaning,
2. Coating,
3. Controlling,
4. Decorating,
5. Disintegrating,
6. Drying,
7. Evaporating,
8. Forming,
9. Materials handling,
10. Mixing,
11. Packaging,
12. Pumping,
13. Separating,
14. Storing,
15. Heat transfer.

We have separated materials handling and pumping, a separation which, at the present moment, seems to me to be of dubious validity.

To get an idea of the number of subsidiary names under one unit operation, take No. 14, *separating*. This is a very large group because a considerable function of the business of manufacturing food consists of tearing down the highly organized structure that constitutes most of our raw material. In many cases the raw material is a finished product from nature, and man desires to separate different parts or components from the original substance.

Under *separating*, come the 23 following names :

- a. Centrifuging,
- b. Draining,
- c. Evacuating,
- d. Exhausting,
- e. Filtering,
- f. Freezing for concentration,
- g. Percolating,
- h. Pitting,
- i. Pressing,
- j. Riffing,
- k. Riddling,
- l. Cleaning,
- m. Sedimenting (settling),
- n. Sifting,
- o. Skinning,
- p. Sorting,
- q. Tabling,
- r. Skimming,
- s. Vacuumizing,
- t. Crystallizing,
- u. Extracting,
- v. Dissolving,
- w. Cutting.

Inasmuch as we are considering the importance of the unit-operation concept, rather than the concepts themselves, we will take up at this point the unit processes. When considering the unit processes of food engineering in which the composition of matter is transformed, one runs into a field in which there is less hope of orderly arrangement of knowledge than exists in the chemical-engineering unit processes. In chemical engineering some of the typical unit processes are nitration, sulphonation, oxidation, reduction, diazotization, saponification, roasting, calcination, and the like. All of them result in fairly clean-cut chemical reactions in which the major product is the substance desired; and in most cases the by-products, if any, are fairly well defined or identifiable substances.

On the contrary, in the unit processes of food engineering there are innumerable by-products of the transformation of matter which are complex, difficult to identify, and often are the very things that render the food valuable from the standpoint of palatability. As an example, take roasting as applied to coffee. I doubt if anyone could state in quantitative chemical terms what green coffee is. Nor is it any easier to identify what roasted coffee is. The same is true of malt.

Apparently, so long as foods themselves consist of mixtures of substances in which the proportions of components of the mixture can be widely varied without ruining edibility of the food, it seems

likely that knowledge of unit processes in food engineering will not be as thorough as the corresponding knowledge of unit processes in chemical engineering. Chemical engineers do not yet make any attempt to classify their unit processes. In that profession, dealing with simpler chemical reactions, if they throw up their hands in despair, what shall we do with respect to transformations of matter in the complex field of biological chemistry? I think anyone may be pardoned for evading any serious attempt at the present time.

Because the term "unit process" is to be used a little later, a number of the industrial scale transformations of matter that deserve to be described as unit processes are listed so that they may be fully understood; for example, *fermentation* which can be subdivided into alcoholic, acetic, citric, lactic, and mixed fermentations such as are found in sauerkraut manufacture.

Hydrolysis, another unit process, is largely used in the manufacture of derivatives of starch including mashing, and dough "fermentation"; or derivatives of sugar; and the clarification of fruit juices. Then there are the results of *heat application* which brings about a change of composition of matter, such as baking, brewing, coagulating, roasting, cooking, boiling, rendering, and refining.

The foregoing list is very far from complete. The items merely typify the sort of process to be found. You will no doubt readily agree that unit processes are oftentimes intimately associated with the chemistry of the given food material to a greater extent than is the case in the corresponding relationship of a unit operation and *any particular food*.

My own concept of food engineering is that it is that branch of engineering which deals with the industrial production of foods and foodstuffs. Food engineering is the application of the principles of engineering, chemistry, physics, and bacteriology to the process. In no way does it displace the work of the practitioner of any of those sciences. Somebody has to translate their work into a plant operation. That somebody must know not only those sciences but also the industrial methods by which scientific knowledge is translated into the best possible industrial practice. He is the food engineer.

Back in 1922, the American Institute of Chemical Engineers proposed and adopted a definition of chemical engineering after many years of attack by the chemists. If I were to paraphrase that definition and apply it to food engineering it would read as follows:

"Food engineering is not a composite of chemistry, mechanical engineering, bacteriology, and physics but is itself a branch of engineering, the basis of which is those unit operations which in their

proper sequence and coördination constitute a food-manufacturing process as conducted on an industrial scale.”

It is only when men are dealing with a food which they have just invented and for which there is no known manufacturing procedure that they naturally think in terms of unit operations and unit processes and try, with an open mind, to find out the best procedure. As soon as the manufacture of a well-known food comes under consideration there is very little of the new method of approach which is so important.

Until better-trained men—men who are trained in what we choose to call food engineering—form a sizable portion of the population of the food-manufacturing industry, technical progress of the industry is going to be an extension of the slow development which has characterized the past. Progress will become much more rapid when all the food industries speak in a common language and study their fundamental problems scientifically instead of haphazardly.

RECENT DEVELOPMENTS IN CANNING TECHNOLOGY WITH REFERENCE TO SPOILAGE CONTROL

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The development of spoilage control in canning has necessarily been slow. Conscious, tangible effort in this direction dates back to the first realization by scientifically trained technologists that certain types of bacteria are associated with spoilage of canned foods and that, in the case of nonacid foods, the significant organisms form spores of such high resistance to heat that temperatures higher than boiling are necessary for their destruction.

The empirical method of Appert defined the basis for the so-called "art" of canning, and the studies of Pasteur laid the groundwork for canning, the applied science, as it is today. It remained, however, for those who possessed collateral talents of scientific training and a working knowledge of food processing to bring definitely to attention of the canning industry details of the mechanism of food preservation by canning. We, therefore, look back to the period around 1895, when Prescott and Underwood in Boston and Russell in Wisconsin reported their studies on spoilage in corn and peas, as the starting point in practical spoilage control. Certainly these investigations centered the attention of farsighted canners on the importance of the new science of bacteriology.

From that time the significance of temperature-time treatment known as the process was gradually impressed upon canners, but from word-of-mouth reports I have received it appears that severe spoilage losses occurred at times.

As time went on and packs grew larger, repeated outbreaks of spoilage caused genuine alarm, and various workers gave their attention to the problem. Among them was Bronson Barlow, who in 1913 was working for his master's degree at the University of Illinois. He investigated at least two outbreaks of spoilage and demonstrated the importance of the thermophilic spore-forming group as causes of spoilage. His descriptions and deductions as set forth in his thesis were well ahead of his time, but unfortunately the information he derived was not for some years brought to attention of the industry in such form that it could be applied in prevention of spoilage.

The third landmark in the evolution of spoilage control was the development by Bigelow in 1918 of apparatus and methods to study

heat penetration in processing canned foods, temperature of pressure kettles, and heat resistance of bacterial spores. These were the beginning of special processing studies which are still conducted under his direction at the Research Laboratory of the National Canners Association.

Spoilage control in the industry as now applied from the standpoint of recent developments may conveniently be discussed under four headings, as follows: the process, control of contamination in canning plants, control of contamination in ingredients, and control of contamination in the raw product.

THE PROCESS

The process is the treatment given to produce effective sterilization; I wish, therefore, to set forth in some detail the basis for present processes as recommended by the National Canners Association.

The processing studies earlier referred to were begun in 1918 and are still in progress. First investigations centered on the development of heat-penetration technique and an analysis of findings. This was followed in 1919 by bacteriological studies directed toward the refinement of procedures for determining heat resistance of very resistant spores and a study of organisms most frequently concerned in spoilage of nonacid products. The first publications on this work came from the National Canners Association in 1920. Shortly thereafter, experimental packs inoculated with spores of known heat resistance were put up for the purpose of testing processes calculated from experimental data. By this means, the validity of calculated processes was established. Bacteriological field studies to determine sources of contamination were undertaken in a small way in 1924, and since 1926 these studies have become a major phase of the general program initiated in 1918.

As knowledge of process requirements developed, process suggestions were made to cover the more important products. There was need, however, for process suggestions to be applied to a wide range of nonacid products, and in 1929 a series of conferences was held which brought together experts in canning technology who had given major attention to the theoretical and practical details of processes.

In 1930, the Board of Directors of the National Canners Association approved for publication the processes to be used for various nonacid products. The Association's processing bulletin, No. 26-L, was issued in that year, and there have been two revisions, the last of which was issued last spring.

As previously indicated, the processes given in this bulletin represent the composite opinion of experts in canning technology. In the

main, these processes are based upon technological data relating to heat resistance of spoilage bacteria in canned foods and upon data pertaining to heat penetration in canned foods. With certain products, such as peas, corn, and spinach, a wealth of information is available regarding the important types of spoilage bacteria, their resistance, and the rates of heat penetration in the respective products. For these products, process calculations based upon mathematical treatment of the heat-resistance and heat-penetration data have been tested by means of large experimental packs put up by commercial methods and inoculated in part with typical spoilage bacteria of significant heat resistance.

For other products less technological information is available, and in some instances process suggestions are based upon analogy with similar products for which heat-resistance and heat-penetration data are available and also on the general experience of the canning industry.

It is understood that changes will be made in process suggestions as new technological developments and new information warrant. The processes are, therefore, not to be regarded as fixed and unalterable, and when further data and information are available, revised editions of the bulletin will be issued.

To the best of our knowledge all processes for nonacid products fulfill the basic requirement that they shall be adequate to destroy *Clostridium botulinum*, which is the most resistant food-poisoning type. It is well known that spores of different strains of this organism will vary in resistance, and it is possible, of course, that the resistance value which has been set may later be revised. The experience of the industry over the past 12 or more years indicates, however, that the resistance value has been set at a safe level.

As a practical matter, pH 4.5 has been taken as the point above which products should be processed under pressure to a degree sufficient to destroy all food-poisoning bacteria. It is true that growth of spores may be irregular in the lower regions of the nonacid range, but even occasional development in growth emphasizes the necessity for a protective process.

The application of this basic requirement has been universal. No distinction has been made from the fact that this or that product has never been involved in food poisoning, nor has there been any geographical distinction. To justify such process differentials would call for convincing data which, so far as I know, nowhere exist.

With respect to newer developments in the field of process investigation, I call attention first to extension of knowledge regarding organisms responsible for spoilage—particularly the “flat-sour” bacteria, *B. steptothermophilus* (Donk), the hydrogen-producing anae-

robes, *Clostridium thermosaccharolyticum* (McClung), and the sulfide-spoilage organism, *Clostridium nigrificans* (Werkman and Weaver). There has been marked progress in improvement of detection media for these types and they can now be isolated at will. Next, attention is called to the intensive field work which aims at the discovery of sources of spoilage contamination and its control. Associated with this, there has been marked improvement of technique for the production of spores for laboratory study and inoculation of experimental packs, as well as for the determination of the resistance of highly resistant spores. Finally, the technique of heat-penetration determination has advanced and the mathematical analysis of heat-penetration and heat-resistance data has developed.

CONTROL OF CONTAMINATION IN CANNING PLANTS

Before entering into discussion of recent advances in control of contamination within the canning plant, it should be emphasized that such control relates principally to the elimination of thermophilic organisms which as a group produce spores of a resistance far beyond that of any types in the category of food-poisoning organisms. To be more specific, attention is directed particularly to the flat-sour organisms, the thermophilic anaerobes (gas-producers), and the sulfide-spoilage bacteria, all of which are thermophilic spore formers. In addition, search has been made for the mesophilic group of putrefactive anaerobes. In the case of acid products, such as tomato juice, these organisms are not of importance, and attention is given to such bacteria as butyric spore formers, the flat-sour spore formers, and nonsporing lactic acid forms.

Our process recommendations, to which reference has been made, may not be effective in the event of abnormal contamination of unusual resistance; and to prevent spoilage in such instances the solution must come through the elimination of contamination at the source.

Prior to the last six or eight years it was the general belief, which has persisted to some extent up to the present time, that the bacterial condition of the raw product was all important. It was assumed, although there appears never to have been any factual data to establish the point, that the important source of all contamination was soil; that the product carried soil contamination to the canning plant; and that if the product was held at the plant before canning, the temperature increased and the organisms multiplied. It was therefore claimed that a product, such as corn, which had been held several hours would require a higher process than that freshly packed. This was our thought in the early days of field investigation, and we expected to find, through culture tests and experimental packs, that

as the canning period progressed spoilage contamination would increase and a given process would be correspondingly less efficient.

In our first venture we were interested particularly in flat-sour organisms. We did not find that these types increased during the canning period; instead, we found the reverse to be true. Flat-sour spores were found in large numbers at the beginning of the day's pack, and the number dwindled as the pack progressed. This was established not only by culture tests but by experimental packs. The answer was quite apparent. There had been overnight development in the canning equipment and the spores served to contaminate the first of the pack. There was subsequent reduction by dilution. This, of course, is no argument to justify the holding of a product before canning. The raw material should be handled with dispatch; otherwise loss of quality is to be expected.

Soil contamination directly transferred to the canning plant through the medium of the raw product is known to be a probable factor in spoilage but only in exceptional cases; this point will be considered later. The holding of such products as peas or corn will not promote the growth of resistant types even though the temperature is measurably increased by respiration of the product. The organisms that grow during the holding period are largely lactic acid producers and other fast-growing, nonspore formers. We have held such products as peas and corn over long periods and we have noted enormous increases in the numbers of vegetative forms, but in no case was there any appreciable increase in spores and usually a reduction occurred. We have heard of instances in which processes have been increased to counter assumed increases in contamination where the product has been held for several hours before it was canned. The application of such process differentials cannot be regarded as sound practice.

As to sources of contamination within the canning plant, we have found that the more complicated the operation the more acute are the spoilage hazards; for example, let us consider contrasting conditions in the canning of corn.

The simplest procedure involves mixing of the corn and brine while cold, followed by a short precook just prior to sealing. Heat is applied only in the filler-cooker which is attached to the filling machine, and this is the only point at which one would be likely to find any overnight increase in numbers of thermophilic organisms.

Going to the other extreme we find corn preheated in mixers, transferred to blending tanks and perhaps storage tanks, then pumped around to the various fillers through a circulating system. In these large heated units there are many points at which thermophilic organ-

isms may develop, and unless proper attention is given to cleaning and cooling the equipment, a spoilage hazard may result. Fortunately, however, reasonable attention will serve to keep the more complicated set-up in safe condition.

It is only a few years since tomato juice became a factor in the market for canned products. Prior to that time, spoilage in tomato products was almost always apparent through the occurrence of swelled cans. We have been told of rare instances of so-called flat-sour spoilage, in which there was acid without gas production, but these were exceptional cases. Shortly after large-scale production of tomato juice began, however, we obtained reports of flat-sour spoilage and samples were submitted to the laboratory which showed this condition. In some instances, there was a measurable lowering of pH; in others, the juice had an "off" or "medicinal" taste. The same general type of organism was found to be responsible and was named *B. thermoacidurans* (Berry). The organism was thermophilic and formed spores, and spoilage by spore-forming organisms is very rare in acid products. Probably because of its acidity the medium was not ideal, and this accounts for certain of the abnormal reactions which were noted.

In tracing a major outbreak of this type of spoilage, again certain elements of equipment were found to be involved, such as the pre-heater, juice extractors, and pumps. Our investigation indicated the elements of equipment which should be subject to special care, and the information was brought to attention of the industry. At the present time spoilage in tomato juice is very rare.

Similar stories could be told with reference to other major products, such as pumpkin, peas, and spinach. In the canning of pumpkin we have relative freedom from spoilage organisms where a simple operation is followed, and we look askance at certain types of wilters, presses, and pumps. In canning peas, blanchers require close attention. All told, our experience has been that while modern equipment is essential to efficient production and maintenance of quality, it does present certain problems in contamination control which must be recognized. Moreover, it has been shown that adequate study will reveal how these problems may be met.

Not many years ago the attention of the equipment manufacturer centered particularly upon invention of devices which would increase production, and he brought forth such equipment as viners, bucket-elevators, and the like. Next was considered the question of quality, and the industry was given improved washers, graders, quality separators, etc. Now, however, the manufacturer is awake to the further requirement that the equipment must be capable of simple and effec-

tive cleaning and, in some instances, sterilization under high pressure. An outstanding example of this is the increasing use of hydraulic elevators for peas, and, more important, tubular blanchers which may readily be cleaned and sterilized under pressure. It is my prediction that during the next few years there will be rapid replacements of the old-type blancher with those of tubular construction. Here again, however, there must be some special study to determine whether one method of handling the tubular blancher is better than another.

We have found that mere inspection of equipment is not sufficient, regardless of the observer's experience and technical ability. We can hazard the opinion that this or that type of equipment, or method of handling, may or may not be satisfactory from the contamination viewpoint, but the only safe method is to investigate actual operations before giving final opinion.

Flume systems find increasing use in the conveyance of peas through the cannery, and there is no question about their advantages. Some systems utilize cold water and we found this year that hot water was used in many of them. In one case where hot water was used, the water was recirculated through the flume and back to a reservoir. The entire system was cleaned during the night, noon, and evening shutdowns; and after being cleaned the reservoir was filled with fresh water. The water was kept at a temperature of 65.6 to 76.7°C. (150 to 170°F.). After each refill, negative counts were obtained for thermophilic spoilage types. As the pack progressed, however, there was an amazing increase in spores until, just before shutdown and cleaning, there was no question about the existence of a spoilage hazard. There was a choice of three remedies in this case: first, to use cold water; second, to use water at 76.7°C. (170°F.) or higher, at which level there would be no thermophilic growth; or third, to use hot water at 65.6 to 71.1°C. (150 to 160°F.) and waste the discharge water. Prior to our tests, however, the original system was regarded as sound.

During the next few years we may look for more and more thought directed to the invention of contamination-proof equipment and the devising of canning systems which will answer production needs without adding any spoilage possibilities. Especially, there will undoubtedly be development of equipment which may be sterilized under high pressure.

CONTROL OF CONTAMINATION IN INGREDIENTS

Obviously, precautions to keep contamination at a low level through the utilization of proper equipment and strict cleanliness would be offset to a marked degree by contamination that may come

with ingredients, and within the past few years it has been impressed upon the industry that certain ingredients are likely to provide spoilage contamination.

Our first appreciation of this fact came through knowledge that sugar may carry spores of thermophilic bacteria and that these spores may serve to seed the canning equipment, contamination in some cases being so high as to be direct causes of spoilage. This problem has been discussed at length at canners' meetings and through publications. By experimental packs we have been able to prove beyond question the relation between sugar contamination and spoilage. Standards have been set up by the National Canners Association and have been widely used by the canning industry and the sugar industry in appraising the bacterial condition of sugar. The sugar industry has met the problem squarely, and the great majority of canners of non-acid products now specify that sugar shall be of such bacterial quality as will meet the N.C.A. standards. The problem has been solved through the cooperation of these two branches of the food industry.

Aside from the sugar problem, we know that other ingredients, such as starch, flour, syrups, and milk powder, may carry spores of spoilage thermophiles. The problem of starch contamination seems to have been solved by leading producers, and sugar standards have been applied to that product. So far as we know, however, nothing has been done to control thermophilic contamination in flour, and serious spoilage has been traced to this source. Therefore we have advised the use of tested starch in place of flour when the substitution can be made without detriment to the product.

Thermophilic contamination of milk powder has been found of importance principally in the preparation of certain soups. From results of study by certain producers of milk powder, however, it appears that this condition can be remedied without difficulty.

In some instances canned products may be used in the preparation of other canned products and are, therefore, themselves ingredients. Here it is important to know whether dormant thermophiles are present because of possibility of spoilage in the finished product, which may provide more favorable environment for growth.

CONTROL OF CONTAMINATION IN THE RAW PRODUCT

We have few established incidents of spoilage directly resulting from soil contamination. In these cases there was a direct mechanical transfer, with no suggestion that the organisms had increased during holding; for instance, in the study of one such case of spoilage in asparagus, it was found that asparagus from certain sections in cer-

tain fields could not be adequately sterilized by the usual heat treatment. Soil tests demonstrated that these condemned sections were infected with thermophilic bacteria. Attention is called to the fact, however, that the cleaning of asparagus presents certain peculiarities.

In the future we may expect to see still further refinement of techniques of heat-penetration and heat-resistance determination and extended consideration of products not at present adequately covered by specific research. Consequently, ideas regarding processing requirements may be modified in the case of certain products.

We may also look for changes in canning equipment, and manufacturers will look to reduction in spoilage contamination as well as to improved quality.

Finally, more and more attention will be given to bacterial condition of canning ingredients. Where experience indicates, purchases will be made on specification as to bacterial quality, and samples of deliveries will be tested by control laboratories.

PRESENT USE AND FUTURE PROSPECTS OF OZONE IN FOOD STORAGE

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Ozone has been used in food storage for many years in America and Europe; conflicting views as to its use have been expressed. The purpose of this paper is to protect it from both overenthusiastic friends and well meaning, but ill-informed, ignorant detractors.

Above 10°C.(50°F.) ozone does not appreciably inhibit growth of bacteria upon food surfaces containing large water content, unless such high concentrations are employed that the accompanying deleterious effect of the strong oxidizing agent vitiates its advantages. It is, however, of value in sterilizing the air, according to Ewell (1936), and in destroying (not simply masking) odors from both good and spoiled food, Teichmann (1932).

The primary purpose of low temperatures in food storage is retardation of physiological or biochemical changes and of growth of micro-organisms inducing decompositions. Protection from serious loss of color and injury to surface taste requires high humidity, and with increasing humidity the growth of bacteria and mold spreads rapidly. Treatment with sterilizing agents, such as formaldehyde, is generally prohibited. Introduction of other gases, such as ethylene, ammonia, and acetaldehyde, either gives little advantage or presents marked disadvantages.

Carbon dioxide, however, at concentrations of five to 20 per cent has a remarkable specific inhibiting effect upon bacterial and mold growth, but it has the disadvantage of the difficulty of securing gas tightness. In storage chambers it usually replaces oxygen to a far greater extent than nitrogen, so that men cannot work in the room. Most large cold-storage rooms must be entered frequently for removal of stored goods or entry of fresh products, thus precluding the use of carbon dioxide.

Air motion is generally of value in keeping down bacteria and mold. It is particularly useful in the case of certain vegetables, especially celery. In the case of meat Loeser (1934) reported that it might actually increase bacterial growth if the humidity is above 85 per cent. It increases the loss by shrinkage and, in the case of beef, very seriously affects the color, reducing the "bloom." A velocity of one meter per second at 6.7°C.(44°F.) and 90 per cent humidity

will impair the color to such an extent that the storage time is reduced to one-half.

The agent most commonly employed against mold and bacteria is ozone, an allotropic form of oxygen having three atoms rather than the customary two. This extra atom is readily released and becomes one of the most powerful oxidizing agents known. Since the residue is common oxygen, there are no objectionable residual gases. The concentrations employed are very low—a few ten thousandths of one per cent—and, therefore, gas tightness of rooms is of no importance. In contrast to carbon dioxide, where the concentration in a gas-tight room remains constant, ozone must be supplied continuously to compensate for decomposition. The cost of production, however, is negligible and the equipment cost is small compared with that of other cold-storage apparatus.

Ozone spontaneously decomposes bimolecularly, particularly if water vapor is present, according to Clement (1904), but at so slow a rate that this cause of disappearance is negligible compared with the monomolecular loss in the presence of oxydizable matter. If k represents the velocity or absorption coefficient, t the time in minutes that ozone has been introduced into an enclosure at the rate of m grams per minute per m^3 , c the concentration attained at this time, and C the final equilibrium concentration, we may apply to all cases the monomolecular reaction equations:

$$\frac{dc}{dt} = m - kc. \qquad C = \frac{m}{k}, \quad c = C(1 - e^{-kt}).$$

For a good egg room, according to Ewell (1930, 1935), k has a value of about .02 and for a full meat room, k is about .1.

There are obvious objections to the use of an oxidizing agent. Foremost is the production of rancidity. At concentrations of 100 to 200 mg. per m^3 or 50 to 100 parts per million, bacon, sausage, lard, cream, butter, dried eggs, mushrooms, meat, and bananas are injured. But at the concentrations required to reduce effectively bacteria and mold, at temperatures below 7°C.(44.6°F.), ozone only produces noticeable increase in rancidity in butter, lard, and fat sausage. At the end of about a week in a continuous concentration of three p.p.m. the surface of butter will show distinct rancidity and also slight bleaching. With prolonged exposure to even these low, continuous concentrations of ozone, a considerable depth of butter will be bleached and turned rancid. Fat sausage becomes rancid in a day or two. Oxidation of pipes and fittings in a cold-storage room in which ozone is used, even continuously at relatively high concentrations (3 p.p.m.), is incon-

sequential. If, however, the doors have rubber gaskets, they may disintegrate to some extent in about six months' time at the ozone concentrations commonly employed if the ozone is continuously present. This is not true of synthetic rubber.

Fortunately, the effectiveness of ozone in destroying bacteria and mold is largely dependent upon the equilibrium concentration. This is attained usually, except in egg rooms, in less than 20 minutes. An equilibrium concentration of about three p.p.m. gives nearly the same germicidal effect whether applied continuously or for two two-hour periods a day or for one three-hour period. With ozone present for so short a portion of the day, if the storage is limited to a few weeks, rancidity appears only in the case of fat sausage, and rubber door gaskets last for over a year. If butter and lard are to be carried many weeks they should be kept in covered containers.

Long exposure to ozone at the proper concentrations for meat rooms, and to a less extent at the concentrations in egg rooms, produces headache and irritation of the mucous membrane of the throat. It is, therefore, customary to introduce ozone into meat rooms only at those hours of the day when men are not working in them for any length of time, and to turn off the otherwise continuous supply of ozone to egg rooms during filling and removal of eggs.

Exhaustive studies of the growth of bacteria, by Schmid (1931), and of mold, by Schwartz and Kaess (1934), on meat during storage and the changes in color (using the Block photometer), by Heiss and Hohler (1933), have been carried out at the Refrigeration Technical Laboratory at Karlsruhe. With a few exceptions, the specimens studied were taken from a freshly killed carcass and handled with extreme care with the result that the initial infection was low and quite constant. The writer has conducted similar tests with many hundred meat specimens procured from commercial meat houses and markets. The results of the two investigations are not always in agreement owing particularly to differences in initial infection. The storage time to the appearance of slime is generally much less with market samples than with the carefully protected Karlsruhe specimens. The time, however, for serious color loss and for visible appearance of mold was often somewhat greater. The writer studied the color changes by comparison with 25 standard colors covering the range from brilliant "bloom" to almost black.

Both bacteria and mold absorb moisture in their growth. The faster growing of the two consumes so much of the available water that growth of the other is checked. Dropping the temperature reduces bacterial growth far more than it does mold development. Below about 3°C. (37.4°F.) mold grows much the faster of the two

unless the bacteria have obtained an advantage by high initial infection. The latter is generally the case with small cuts of meat, owing to extensive handling and exposure to infection, and it is also often the case with large pieces of meat in Europe, according to Loeser (1935). In America the initial infection of large cuts is generally so moderate that spoilage is due to mold rather than to bacteria.

Above about 3°C. with all beef, and at lower temperatures when badly infected, surface spoilage is due to bacteria rather than to mold.

The most careful and comprehensive study of the effect of ozone upon bacteria on the surface of beef is that of Kaess (1936) at Karlsruhe. The meat specimens used had initial infections between 10^2 and 10^6 bacteria per cm^2 . His results are in substantial agreement, so far as they cover comparable specimens, with those of the British Food Investigation Board and with my own. Perhaps the most interesting discovery of Dr. Kaess was that the bacteria upon beef become acclimated to ozone, and after a few days the presence of ozone increases the rapidity of growth. He, therefore, advises (for a temperature of 3°C. and 90 per cent humidity) the application of ozone for only three or four days. Inasmuch as the meat in a warehouse or refrigerator is constantly changing, such a procedure would be impracticable. At this temperature and this humidity, meat with low initial infection (not over 10^3 bacteria per cm^2) without ozone will carry six days without bacterial spoilage, but serious color loss occurs in five days and mold usually appears in about four days. If meat is badly infected bacterially when placed in the refrigerator, bacterial spoilage may occur in two or three days. A concentration of three to five p.p.m. for a three-hour period each day will not appreciably hasten color loss and will inhibit mold and bacteria until, at about five days, loss of color has seriously impaired the appearance of the beef.

A heavy growth of mold, or "whiskers," appears in the course of a few weeks upon beef free from high initial bacterial infection at temperatures between 1 and 3°C. (33.8 and 37.4°F.) and a humidity of 90 per cent or greater. Ozone is much more effective in checking mold than bacteria; and with "clean" beef, at these temperatures, it will invariably increase the length of time several hundred per cent before the visible appearance of mold, from about two weeks to eight weeks. The best procedure is a two-hour ozonization twice a day, once in the afternoon, or when the workers have left, and the second in the early morning before the cutters and porters arrive. The equilibrium concentration should be between two and a half and three p.p.m., according to Ewell (1935). He also stated that if mold appears

owing to contamination from the outside or interruption in the supply of ozone, the following ozonization (two or three p.p.m.) will remove a day's growth. It is interesting to note that even though there is no visible mold to compete with bacteria for the available moisture, the ozone keeps down the bacterial content, if initially low, to a saleable limit. The natural color of cut surfaces of large pieces of beef, free from serious initial infection, lasts much longer than with small specimens under similar conditions, owing undoubtedly to the greater amount of moisture available from the interior. The writer has investigated very carefully and extensively the growth of mold on beef in rooms supplied with ozone but has never detected any evidence of acclimatization such as Kaess found with bacteria and such as was indicated in some experiments of Heiling and Scupin (1935) with fruit mold.

Ozone is relatively unimportant in freezer storage since bacteria and mold growth is small compared with that at cooler temperatures, i.e., above freezing. It is often of great value in sweetening freezer rooms which have acquired a bad odor.

Small fresh fruits, such as strawberries, raspberries, currants, and grapes (particularly sweet wine grapes), are especially subject to mold. Their storage period may be doubled by two or three p.p.m. of ozone applied continuously or for several hours each day, provided they are not so closely packed as to hinder access of ozone. Apples carry at a proper temperature and high humidity very satisfactorily without ozone, but the length of storage, especially of the more delicate and valuable varieties, may be prolonged many weeks by a similar use of ozone. Both English investigations, by Moran (1935), and my own show that such concentrations have no injurious effect upon apples. The use of ozone in apple storage destroys the unpleasant "dead" odor often noticeable, but it also produces an inoffensive cloud throughout the entire room, owing to oxidation of vapors given off by the apples.

It seems definitely established by Kochs (1931) that aromatic fruits, such as strawberries, acquire a slightly richer characteristic odor and aroma when ozone is present.

Several reliable investigators in this country and in Germany have reported that both butter and cream acquire an improved flavor after a few days in an ozonized atmosphere. The storage of fresh fish is moderately improved by ozone. The carry of many vegetables—*asparagus*, *beets*, *cabbage*, *carrots*, *cauliflower*, *cucumbers*, *kohl-rabi*, *lettuce*, *radishes*, and *tomatoes*—is lengthened by a moderate supply of ozone, according to Kochs (1931) and Beckel (1927).

Ozone has achieved its greatest success in egg rooms, where a high humidity is necessary to reduce shrinkage owing to evaporation. The growth of mold naturally increases rapidly with increase in water vapor in the storage room. A minimum continuous concentration of .6 p.p.m. of ozone is necessary to prevent mold growth if the eggs are relatively "clean." For "dirty" eggs and to remove mold already present, a concentration of 1.5 p.p.m. is desirable. In a storage room filled with clean eggs with suitable aisles and overhead ducts a concentration of 1.5 p.p.m. in the aisles will assure that the concentration will not decrease below .6 in the center of the piles. Continuous introduction of ozone is preferable to introduction for a few hours each day. Under such conditions eggs kept at -0.6°C. (31°F.) with a humidity of around 90 per cent will, after eight months' storage, be indistinguishable from eggs a few days old when eaten raw or cooked, as shown by Horne and Pennington (1924). My observations, as well as those of Moran (1935), have shown that a continuous ozone concentration as high as 3.5 p.p.m. for months will not injure eggs.

The use of ozone has been very seriously handicapped by lack of scientific quantitative methods both in research and in practice. Without knowledge of the actual concentrations of ozone employed the results of tests of its use are of little value.

A quantitative chemical determination of the ozone content of a known volume of air in a storage room requires apparatus, skill, and time not always at hand, but necessary for accurate results. There are now available special potassium iodide-starch test papers with standard colors. A test paper is hung where the concentration of ozone is desired and the minutes after moistening are counted until the color of the paper matches one of the three standard colors. From a table of times-colors the concentration is determined with an accuracy of about 10 per cent.

The design of ozonators, with a very few exceptions, reveals an almost complete ignorance of the principles of production of ozone by the ionization of oxygen despite careful formulation in the papers of Warburg (1905), von Wartenberg (1925), Ewell (1906), and others. I believe there are only two manufacturers who produce entirely satisfactory machines and these only in the larger models. One other company puts out a reasonably efficient and satisfactory product. The remaining score or more of ozonators for sale in Europe, England, and America are inefficient in production of ozone, very unreliable in operation, and require frequent repairs or overhauls. The poorest ozonators are the ones most widely advertised with the crudest exaggerations of the value of ozone.

The introduction and distribution of ozone in cold-storage rooms is often very inefficient. Most serious is the failure of all but a few of the leading cold-storage plants to determine periodically the actual concentration of ozone in the different portions of each room. Quantitative determination is often limited to the sense of smell which has been found subject to many errors. With the poor quality of most ozonators, ignorance of the actual concentration of ozone obtained and often poor distribution, is it not natural that many cold-storage plants still use very little ozone while some others which do employ it are skeptical about the value?

At the present time the majority of large egg-storage rooms throughout the world are provided with ozone-supplying equipment. In this country a minority have satisfactory ozonators and proper distribution ducts or fans, and by quantitative tests assure the necessary ozone concentration. The proportion of those in England and on the Continent which maintain a proper ozone concentration is much less. Within the past year there has been a great improvement in Australia and in other parts of the Southern Hemisphere. Most large meat rooms on the Continent use ozone. Often adequate concentrations (a few p.p.m.) are maintained in the initial cooling room during the first few days after slaughtering, but rarely is a proper amount supplied the storage rooms. A few meat rooms in this country employ ozone, and some use it in fruit and vegetable rooms.

The experience of the great egg warehouses which use ozone properly is gradually compelling imitation by their competitors. The enhancement of value of meat, particularly beef, by ripening, aging or tendering, is now generally appreciated in England and is being adopted slowly in this country. The customary method of ripening in use at the present time is prolonged storage at a few degrees above freezing. This results in losses owing to invasion of the tissues and consequent taint in taste and color. These losses may be largely prevented by proper ozonization since proper ripening is independent of mold growth.

No domestic refrigerators are provided with ozonators, but this offers a possible field for development. The advantages which have been already described can be secured in small units also if properly designed equipment is made available. There will undoubtedly be reliable small ozonators for small meat rooms and domestic refrigerators in the near future. Experimental models have been thoroughly tested and found as satisfactory as the best large ozonators.

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CAROTENE AND ASCORBIC ACID CONTENT OF FRESH MARKET AND COMMERCIALLY FROZEN FRUITS AND VEGETABLES¹

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The present status of our knowledge of the stability of vitamins A and C in fruits and vegetables when subjected to varied conditions of storage and freezing are given here in the form of a review of investigations and original data. It is hoped that the facts presented will give a clearer conception of the effect of certain environmental factors on the vitamins. Frozen fruits and vegetables are becoming more and more widely consumed and form an important addition to the human dietary. Retention of the vitamins in these processed foods is of the greatest health interest.

Until recently it has been impossible to evaluate exactly the vitamin values of foods obtainable on the market because the standard tests using the biological or animal-survey methods could not always detect variations such as are caused by maturity, variety, locality, or temperature of exposure.

With the discovery by Tillmans, Hirsch, and Hirsch (1932) of the rapid chemical method of determining vitamin C and its rapid development and improvement by many laboratories, investigations on the effect of environmental conditions, such as storage, processing, and cooking, have been greatly stimulated. Similarly, recent development of spectrophotometric and chemical methods for the determination of vitamin A and carotene has led to a renewed interest in the vitamin A content of foods and the effect of various factors involved in food distribution and preparation of this vitamin. Vitamin A, however, has been far less concerned in food research during the past few years than vitamin C. Reviews of quantitative data on the occurrence of vitamins in fresh and processed foods have been published by Fellers (1936) and by Daniel and Munsell (1937). Because of the newness of successful freezing methods, relatively fewer investigations have been conducted on the effect of freezing than on the various heat treatments.

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CAROTENE CONTENT (VITAMIN A) OF FRUITS AND VEGETABLES

The approximate contents of vitamin A in fruits and vegetables in both the fresh and frozen states are shown (Tables 1 and 2). Some of the data on fresh fruits and vegetables are taken from the literature, particularly from Daniel and Munsell (1937); on the whole, the data on frozen vegetables and fruits are our own. Of course, no direct comparison can be made between these values in most cases since the samples are not the same, yet they show rather consistently that commercially frozen foods retain substantially unimpaired their

TABLE 1
Vitamin A (Carotene) Content¹ of Fresh and Frozen Fruits

Fruits	Fresh	Frozen
	<i>I. U. per 100 gm.</i>	<i>I. U. per 100 gm.</i>
Apples.....	58-147	36
Bananas.....	370-147 ²
Blackberries.....	280-560 ²	No change ²
Blueberries.....	100	110
Cherries.....	200-1,600 ²	700-1,600 ²
Cranberries.....	20	20
Grapes.....	15-90 ²	No change ²
Grapefruit.....	0 ²
Oranges.....	65 ²
Peaches (yellow).....	300-3,000	2,000
Pears.....	8-20 ²
Pineapple.....	100	55 (grated)
Prunes (dried).....	1,400-3,460	2,600 (pulp)
Red raspberries.....	520
Strawberries.....	740
Tomatoes.....	500-3,000 ²	No change ²
Youngberries.....	460

¹ Calculated from .6 microgram of carotene equals one International unit. ² Data not ours.

original vitamin A content after freezing. Since these foods are stored at very low temperatures and protected from light, there seems little likelihood that serious losses in vitamin A occur in frozen foods.

The method used in making the carotene determinations was essentially that suggested by Russell, Taylor, and Chichester (1935) as modified by DeFelice and Fellers (1938).

No apparent loss is indicated in vitamin A content when some fresh fruits are frozen (Table 1). The data on fresh fruits are taken from Daniel and Munsell, but those for the important fruits—strawberries, raspberries, and youngberries—are lacking.

In explanation of these data certain carotenoid pigments, namely alpha, beta, and gamma carotene and the xanthophyll pigment, kryp-

toxanthin, can be converted into vitamin A in the liver. They are, therefore, called precursors of vitamin A. Beta carotene is said to have the greatest potency while the alpha and gamma forms are claimed to have half as much. This is being worked on in many laboratories simultaneously. The present paper assumes that all the carotene is beta carotene and it is indicated as International units of

TABLE 2
Vitamin A (Carotene) Content¹ of Fresh and Frozen Vegetables

Vegetables	Fresh	Frozen
	<i>I. U. per 100 gm.</i>	<i>I. U. per 100 gm.</i>
Asparagus.....	370-1,400	700
Snap beans (green).....	580-1,400	5,400
Snap beans (wax).....	1,240
Beets.....	0 ²	Trace
Broccoli.....	1,300-6,900 ²	4,680
Brussels sprouts.....	290 ²	2,200
Cabbage.....	20-120 ²	300
Carrots.....	1,700-8,400	3,500
Cauliflower.....	70 ²	260
Celery (bleached).....	0-23 ²
Celery (green).....	900-1,300 ²
Sweet corn (Golden Bantam).....	2,500	2,600
Endive.....	27,000 ²
Kale.....	45,000 ²
Lettuce (head).....	230-700
Lima beans (green).....	2,000	1,800
Okra.....	840 ²
Onions.....	0 ²
Green peas.....	600-3,300	4,800
Sweet peppers.....	800-7,700
Potatoes.....	50 ²
Pumpkin and squash.....	2,800-20,000	18,400
Rhubarb.....	Trace	1,300
Rutabaga (white).....	0 ²
Spinach.....	6,600-25,000	30,000
Sweet potatoes.....	1,000-7,000 ²
Turnips.....	0-40

¹Calculated from .6 microgram of carotene equals one International unit. ²Data not ours.

vitamin A (one I. U. equals .6 gamma of beta carotene). The feeding tests indicate that no single conversion factor can be applied to the carotene value to convert it directly into vitamin A. Such factors will be worked out for each vegetable in the near future.

Fifteen frozen products of the 27 vegetables listed were examined for carotene content. These were all commercially frozen vegetables packed in wax-paper packages containing from one to two pounds

TABLE 3
Vitamin C Losses During Ordinary Marketing

Vegetable	Yearly av. wholesale market	24 hours later ¹ at 21.1° C. (70°F.)	48 hours later ¹ at 21.1° C. (70°F.)	Total loss	Average as purchased					
					Summer			Winter		
					Max.	Min.	I. U. per oz.	Max.	Min.	I. U. per oz.
Broccoli.....	I. U. per oz. 77.5 ²	I. U. per oz. 60.0	I. U. per oz. 50.0	pct. 35.5	I. U. per oz. 80	I. U. per oz. 52	I. U. per oz. 99	I. U. per oz. 60.0		
Spinach.....	35.0	20.0	18.5	47.0	53	12	65	29.0		
Peas.....	15.5	14.8	14.0	9.6	18	14	16	12.5		
Asparagus.....	12.5	10.0	10.0	20.0	12.5 ³	9	15 ⁴	12.0		
Snap beans.....	10.0	8.5	7.5	25.0	12.5	9	11	5.7		

¹ At 2.2°C.(36°F.) or lower no loss would have occurred. ² Vitamin values expressed in terms of International units of vitamin C per ounce of product. One International unit equals 0.05 milligram of ascorbic acid (vitamin C) per gram of product. ³ Average for three months—May, June, and July. ⁴ Average for two months—March and April

of the edible product. It will be noted that in many cases the results are higher than the vitamin A values given in the literature for vegetables. It is apparent that freezing causes no measurable loss in vitamin A activity (carotene) of vegetables if they are kept in a solidly frozen condition.

VITAMIN C CONTENT OF FRESH AND FROZEN FRUITS AND VEGETABLES

A comparison is provided between the fresh products in and out of season with the frozen products commercially prepared at several different packing locations on the basis of examinations after six to 12 months of storage (Tables 3 to 12).

All data are presented on the fresh or frozen basis, respectively, and not on the cooked, since recent information indicates that although there is not necessarily appreciable loss of vitamin C content of vegetables on cooking, a large percentage usually passes into the cooking water as do some of the sugars, proteins, and minerals. Until cooking instructions are better developed so as to indicate how best to cook both fresh and frozen vegetables to preserve a maximum amount of their vitamin C content, it seems inadvisable to publish data concerning the cooked products. Eddy, Kohman, and Carlsson (1926), McHenry and Graham (1935), and others have reported from 40 to 75 per cent loss of vitamin C during cooking. Fenton, Tressler, and King (1936) indicated a destruction of vitamin C of only seven to 10 per cent after 15 to 16 minutes boiling, but an apparent additional loss of 48 to 53 per cent was found intact in the cooking water.

The Bessey and King method (1933), using trichloroacetic acid at a pH of .75 to 2.0, was used except for snap beans for which the Mack and Tressler (1937) method of extracting with five per cent sulphuric and two per cent metaphosphoric acid was employed. Each product was tested for the presence of dehydro-ascorbic acid but since the vegetables had all been blanched, none was found. Likewise none was found in the fruits examined although these had not been blanched.

Five vegetable products are listed in the order of their respective vitamin C potency both as purchased and after holding 24 and 48 hours at 21 to 22°C. (69.8 to 71.6°F.). (Table 3.) It is evident that losses are in the order of maximum loss: spinach, broccoli, snap beans, asparagus, and peas. Additional data on the seasonal variations in vitamin C content of fresh market vegetables have been recently published by Feener, Palmer, and Fitzgerald (1937).

Data are presented on the ascorbic acid content of a commercially frozen spinach selected at random out of a cold-storage warehouse

(Table 4). For comparative purposes monthly values for fresh Boston market spinach are presented, averaged from weekly analyses. It will be seen that the ten samples representing five different days' production of the fall pack of Factory I varied less than the fresh spinach did from month to month and was from 20 to 50 per cent higher than July and September fresh spinach. The frozen spinach averaged .33 mg. per gm. compared with an average of .36 mg. per gm. for fresh spinach based on weekly titrations over the period of one year. In spinach frozen in Factory II in the spring of the same year the values ran considerably higher than either the fresh market or

TABLE 4
Variations in Ascorbic Acid Content of Fresh and Frozen Spinach

Month	Boston market	Factory I	Factory II			
	Fresh	Va. Savoy type, frozen	Nobel types, frozen			
	Ascorbic acid	Ascorbic acid	Ascorbic acid			
	<i>mg. per gm.</i>	<i>mg. per gm.</i>	<i>mg. per gm.</i>			
Jan.....	.35 (Texas)	.41 .41	.67	.86 ¹		
Feb.....	.38 (Texas)	.20 .26	.58		
March.....	.36 (Texas)	.20 .26	.47	.75		
April.....	.41 (Va.)	.27 .29	.45	.61		
May.....	.47 (Mass.)	.22 .29	.58	.86	.71	.71
June.....	.21 (Mass.)	.29 .29	.52	.65		
July.....	.15 (Mass.)43		
Sept.....	.14 (Mass.)		
Oct.....	.41 (Mass.)		
Nov.....	.68 (Va.)		
Dec.....	.41 (Texas)		
Average.....	.363360		

¹ Duplicate samples from different days. ² Six different packages from the same day's production.

the other frozen samples produced in Factory I, having an average of .60 mg. per gm., almost twice the amount of either.

Frozen samples of green beans contained about one-half the vitamin C of fresh market beans (Table 5). Since the above data were taken, green beans have been packed in Factory I ranging from .10 to .15, four commercial samples averaging .13 mg. per gm.

Thus it is seen that the same location which provided one product relatively low in vitamin C can provide another which compares favorably with the fresh vegetable available on the market.

Frozen broccoli, it is indicated, can be a far better source of vitamin C than fresh market broccoli (Table 6). Of course, this is relative because fresh market broccoli is still one of the very best sources

of vitamin C, ranking ahead of the citrus juices and being equalled only by a very few vegetables, such as green peppers and Brussels sprouts.

TABLE 5
Variations in Ascorbic Acid Content of Fresh and Frozen Green Beans

Month	Boston market, fresh			Factory III, frozen	
	Ascorbic acid	Variety	State	Ascorbic acid	Variety
	<i>mg. per gm.</i>			<i>mg. per gm.</i>	
Jan.....	.13	Bountiful	Fla.	.112	Bountiful
Feb.....	.12	Bountiful	Fla.	.07	Bountiful
March.....	.11	Bountiful	Fla.	.07	Bountiful
April.....	.14	Bountiful	Fla.	.05	Bountiful
May.....	.18	Bountiful	Fla.	.06	Refugee
June.....	.15	Bountiful	Fla.	.07 .06	Refugee
July.....	.13	Bountiful	Mass.	.04	Refugee
Aug.....	.12	Bountiful	Mass.	.05	Ky. Wonder
Sept.....	.14	Bountiful	Mass.	.06 .07	Ky. Wonder
Oct.....	.18	Bountiful	Md.	.06 .07	Ky. Wonder
Nov.....	.10	Bountiful	Fla.
Dec.....	.10	Bountiful	Fla.
Average.....	.135			.065	

The lack of uniform checks (Table 7) probably indicates an error in sampling of the asparagus. Representative samples of fresh vegetables are extremely hard to obtain. A storage temperature of 1.1°C. (34°F.) appears to cause a retention of from 50 to 100 per cent of

TABLE 6
Variations in Ascorbic Acid Content of Fresh and Frozen Broccoli

Month	Boston market, fresh		Factory III, frozen	
	Ascorbic acid	State	Ascorbic acid	
	<i>mg. per gm.</i>		Buds	Stems
			<i>mg. per gm.</i>	<i>mg. per gm.</i>
Jan.....	1.22	Texas	1.04 ¹	1.49
March.....	.80	Texas	.57	1.28
May.....	.90	Calif.	.67	1.10
June.....	.67	Mass.	1.10	1.17
Oct.....	1.12	Mass.	1.24	1.43
Nov.....	1.08	Calif.	1.08	1.13

¹ Each sample from a different day's production.

the ascorbic acid content. It gives definite indication, however, that the freshness of asparagus affects the vitamin C potency of the resultant product very markedly. It is also seen that overmaturity, which may be caused by holding after cutting, may also be a deleterious

factor. Fellers, Young, Isham, and Clague (1934) found freezing of asparagus caused no loss in vitamin C content.

With the exception of the peas from Mexico a 10 to 40 per cent difference between the fresh and frozen peas is apparent (Table 8).

TABLE 7
Variations in Ascorbic Acid Content of Fresh and Frozen Asparagus

Date	Fresh product			Frozen product	
	Freshly cut—ascorbic acid	Stored 24 hours—ascorbic acid	Temperature	Date	Ascorbic acid
	mg. per gm.	mg. per gm.	°F.		mg. per gm.
May 23.....	.44	.20	35	April 28.....
May 27.....	.31	.32	34	May 1.....	.24
May 30.....	.22	.09	45	May 4.....	.21
June 2.....	.18	.19	35	May 10.....	.23
June 5.....	.24	.12	36	May 13.....	.18
June 9.....	.28	.11	34	May 16.....	.32
11	52	May 20.....	.09 ¹
11	52	May 24.....	.09 ¹
				May 27.....	.15 ¹
				June 3.....	.15 ¹

¹Overmature.

Since some packing locations may have a relatively short and hot season it is not unreasonable to suggest that rapid changes in maturity of peas have a great bearing on the vitamin C content. Rapid handling is also required to prevent losses at high temperatures. It is

TABLE 8
Varietal and Seasonal Differences in Ascorbic Acid Content of Peas

Source	Season	Number of samples	Ascorbic acid
			mg. per gm.
Mexico.....	Jan. to March	33	.28 ¹
California.....	April to June	54	.26 ¹
Wash.-Oregon.....	July and Aug.	39	.25 ¹
California.....	Sept. to Dec.	86	.26 ¹
Factory I.....	June	22	.15 ²
Factory II.....	June	27	.22 ²
Factory III.....	July	28	.17 ²
Factory IV.....	July	19	.18 ²

¹Fresh. ²Frozen.

interesting to note that Fenton and Tressler (1938) state, "Cooking has great bearing with a 59 and 44 per cent retention of vitamin C in cooked frozen and fresh peas respectively," leaving about the same potency "as served" between fresh and frozen. The Factory II frozen

peas incidentally are the equal of the same peas available on the fresh market in Boston after transshipping across the continent under refrigeration. On the other hand, Fellers and Stepat (1936) demonstrated a loss of 50 per cent in the vitamin C content of peas shipped in the pod in iced hampers in from 24 to 48 hours, while the loss owing to freezing operations is only about 20 per cent.

Lacking fresh and locality data for comparison, data are given to show that the inclusion of white or pale lima beans lowers the vitamin C content of a sample markedly (Table 9). The frozen prod-

TABLE 9
Variations in Ascorbic Acid Content of Frozen Lima Beans

Date	Green	Green and white	
	<i>mg per gm.</i>	<i>mg. per gm.</i>	
July 2.....	.22	} 5 to 15% white
July 6.....	.22	.19	
July 10.....	.26	
July 14.....	.25	.23	
July 17.....	.24	
July 21.....	.25	} 5 to 15% white
July 24.....	.26	.18	
July 29.....	.24	.19	
Aug. 2.....	.28	.24 (—5% white) ; .14 (—25% white)	
Aug. 8.....	.26	.21	} 5 to 15% white
Aug. 11.....	.25	.21	
Aug. 14.....	.28	
Aug. 17.....	.23	
Aug. 20.....	.24	

uct is a good source of vitamin C and remarkably uniform when the green beans alone are considered. The green and white mixture, ordinarily running about five to 15 per cent whites, has about 20 per cent less vitamin C, while the 25 per cent white mixture has about 40 per cent less vitamin C than the green beans. Fellers and Stepat (1936a) showed that green lima beans shipped in the pod in iced hampers lost 30 per cent of their vitamin C content. Mature lima beans contained only a trace of vitamin C.

Lacking comparative data on fresh market corn, data are presented (Table 10) for comparison (Table 11). The average vitamin C content of the samples shown (Table 10) is .083 mg. per gm. Dunker, Fellers, and Fitzgerald (1937) found the ascorbic acid content of raw, fresh sweet corn to be .06-.14 mg. per gm. with a mean value of .09 for sweet corn grown in Amherst, Massachusetts. The vitamin C content is affected but slightly by storage in the husk or by freezing.

The variations in vitamin C content are probably due more to state of maturity than to differences in processing (Table 11). The average of the 21 samples for comparison with the fresh samples'

TABLE 10

Variation in Ascorbic Acid Content of Sweet Corn¹ During the 1936 Season

Date harvested and examined	Titration value ascorbic acid
	<i>mg. per gm.</i>
Aug. 15.....	.0897
Aug. 17.....	.1156
Aug. 27.....	.0972
Sept. 4.....	.0652
Sept. 10.....	.0779
Sept. 18.....	.0948
Sept. 26.....	.0780
Oct. 1.....	.0611
Oct. 6.....	.0661

¹Grown at Amherst, Mass.

.083 mg. per gm. of the previous table is .095. Of course, the variety and location are both different but it must not be forgotten that in the case of corn, at least, there is no substantial loss in processing.

TABLE 11

Variation in Ascorbic Acid Content of Frozen Sweet Corn From Factory III

Date	Whole grain	(On cob
	<i>mg. per gm.</i>	<i>mg. per gm.</i>
Aug. 22.....	.11
Aug. 25.....	.096
Aug. 28.....	.085	.095
Aug. 31.....	.103	.12
Sept. 3.....	.095	.115
Sept. 6.....	.085	.09
Sept. 9.....	.09
Sept. 12.....	.10	.10
Sept. 14.....	.07	.095 ¹
Sept. 17.....	.075	.09 ²
Sept. 20.....	.08	.125 ¹
Sept. 24.....	.085	.095 ²
Average.....	.090	.095

¹Somewhat immature. ²Immature.

The limited data on certain other commercially frozen fruits and vegetables (Table 12) show the amounts of both the ascorbic and dehydro-ascorbic acids. It will be noted that significant amounts are not present. This confirms the experience of others that adequately

blanched frozen products do not exhibit oxidation to dehydro-ascorbic acid during low-temperature storage.

Frozen Brussels sprouts are shown to be an excellent source of vitamin C, practically the equivalent of broccoli. Half the vitamin C of frozen pineapple is in the juice extracted when the sugar is added. Again the matter of location and variety enter in, as illustrated in the comparison of the strawberry samples where a 44 per cent difference exists between two locations.

TABLE 12
Dehydro-Ascorbic Acid in Certain Frozen Fruits and Vegetables

Product	Locality	Number of samples	Ascorbic acid	
			Before reduction	After reduction
			<i>mg. per gm.</i>	<i>mg. per gm.</i>
Brussels sprouts.....	Factory II	2	.82	.83
Cauliflower.....	Factory II	6	.28	.29
Pineapple (juice).....	Factory VI	4	.10	.11
Pineapple (flesh).....	Factory VI	4	.09	.10
Green beans.....	Factory I	2	.12	.13
Strawberries.....	Factory V	3	.21	.23
Strawberries.....	Factory II	3	.13	.13

SUMMARY AND CONCLUSIONS

Vitamin A values are given for frozen fruits and vegetables in terms of total extraction value of carotene. Preliminary comparative feeding tests indicate that the frozen products retain practically all their vitamin A during processing and freezing. Storage at low temperature in a practically impervious package out of contact with ultra-violet light indicates that conditions of marketing frozen foods are conducive to complete retention of vitamin A up to the time of delivery to the consumer.

Vitamin C values are given for numerous commercially frozen fruits and vegetables with comparisons provided in many cases with the fresh market products available throughout the year in the same market. The data indicate that climatic, soil, and possibly other factors besides maturity have an important bearing upon vitamin C content of both fresh and frozen foods. They also show that a place which produces a very low vitamin C potency in one product may produce another product with an exceptionally high value. With this knowledge it may be quite possible for a packer with a number of plants to arrange to pack products having a maximum content of vitamin C. Insufficient evidence is available to make such a statement positively, as the accumulated evidence of several years will be

necessary to provide the necessary background. At least, the data here presented will serve as a basis for further investigations in this field.

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SPECTROSCOPY IN FOOD RESEARCH

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The spectroscope is being used more extensively in testing and control of foodstuffs and their containers, as research workers realize more fully the importance of substances which may be injurious or beneficial in minute quantities. The outstanding advantages of spectroscopic methods of analysis are their great sensitivity and the small amount of sample required to carry them out.

Lead determinations in condensed milk give one example where these methods have proved useful. Using the spectroscope, comparison runs of different brands can easily be made from time to time, and the lead content usually found to vary between three and 20 parts per ten million.

Chocolate manufacturers who wish to know the metallic content of their product find the spectrograph useful. The copper content of cranberries from bogs which have been sprayed repeatedly for insect control is of interest to the state and Federal governments, and spectrographic methods have lent themselves ideally to this purpose. Arsenic and lead on sprayed fruits can readily be measured spectroscopically; ashed apple cores are being burned in the electric arc in increasing numbers for analytical purposes. The metallic content of beer kept in cans and in bottles has also been determined spectroscopically.

The spectroscope is being used for analysis in two distinct ways: in the first, small samples of the materials to be analyzed are burned in an electric arc or spark and the light they emit is broken up into a spectrum which is observed visually or photographically; in the second, a method applicable only to substances which are to some extent transparent, light is sent through a thin layer of the material and the wave lengths which have been absorbed are measured. The first method detects the elementary atoms present in the sample, no matter in what form they may be; the second is especially applicable to analysis for complex and organic molecules.

There are many types of spectroscopes; the simple instrument on a tripod that has been used for so many years in flame tests is hardly representative. The cost of a modern installation for spectroscopic analysis of materials may run from two thousand to five thousand dollars or even more. Most firms and laboratories which have installed spectrographs find them very satisfactory, and usually report that

they have saved their cost many times over. In the few cases in which disappointment has been reported it has usually been traceable to the use of unsuitable equipment, ordinarily purchased cheaply on the over-optimistic supposition that any spectrograph can solve any problem. For absorption measurements and for the detection and analysis of elements with simple spectra, such as copper, zinc, tin, the alkalies, and the alkaline earths, an investment of two thousand dollars may suffice; but for general work the more expensive equipment is practically a necessity. One large firm which was particularly impressed with the possibilities of spectrographic analyses invested fifty thousand dollars in equipment and reports that this investment paid for itself in a short time.

A little defining of terms may be useful at this point. At a recent spectroscopy conference representatives of one company announced that they had taken 30,000 spectrograms. A reporter confused spectrogram with spectrograph and wrote in his paper that this firm had purchased 30,000 spectrographs, which caused some perturbation among sales managers. A spectroscope produces the spectrum of any light which passes through it, but when it records this on a photographic plate the result is called a spectrogram, and the spectroscope may be called a spectrograph. The man who works with all these things is a spectroscopist, though nowadays in industry he is likely to call himself a spectrographic engineer.

The greatest advantages of the spectrographic method arise in qualitative analyses. Each chemical element has its own typical spectrum lines, of which only two or three are needed to determine positively their parent atoms; and these lines can be identified by merely measuring the positions of lines in the spectrogram. The operator need merely photograph the light emitted when he burns a small quantity of the sample and then look to see what spectrum lines have appeared, a great simplification over wet methods in which much time must be spent in eliminating substances by groups.

A limitation of emission analysis is that it detects only atoms, since the molecules are largely dissociated in burning. Negative radicals usually do not show up at all; this is particularly true because certain common elements give no lines suited for ordinary spectrographic analysis. Although some 70 of the elements can be detected without difficulty, the gases, the halogens, carbon, sulfur and selenium, and the four heaviest atoms are not well handled by the common spectrographic methods. Special methods can be devised for them, however.

Three milligrams of material will ordinarily suffice to show all of the 70 detectable elements which are present in a sample in concentra-

tions as great as one thousandth of one per cent. While the elements vary greatly among themselves in sensitivity of response, many of the metals can be picked up in quantities as small as 10^{-8} grams.

This great sensitivity suggests a method of internal branding of food products which is being used increasingly. The manufacturer of a material, chewing gum for instance, wants to be able to determine whether a particular sample which may be under suspicion is really his, so he puts into all of his output a small percentage of some harmless metallic material which is not ordinarily present in chewing gum. By using three or four such spectroscopic indicators in various combinations he can even date his output. Since his competitor does not even know that he is using these "branded atoms," much less what they are, the manufacturer has a definite advantage in keeping track of his product.

Common spectrographic methods for qualitative analysis are so satisfactory that the possibility of increasing the sensitivity of detection of this method is frequently overlooked. By moving the photographic plate parallel to the spectrum lines during an exposure, or by taking successive exposures at short intervals during the burning period of the material so as to take advantage of fractional distillation in the arc, sensitivity can often be increased many fold. Thus magnesium and sodium lines may appear only at the beginning of the burning period and gold lines only at the end; in the ordinary method these in very small concentrations might readily be lost in the general background of lines.

The spectrograph, unsurpassed for qualitative analysis, can also be used for quantitatives. Here it has certain limitations which are, however, more than offset by its advantages. At concentrations greater than a few per cent the spectrographic method is considerably less precise than chemical wet methods, but at low concentrations it is far more precise. It is a null method which must be calibrated in terms of samples of known or prepared concentration. Known and unknown concentrations of material are compared through the intensities of some of the spectrum lines of the elements in question. Fortunately, in addition to interpolating between known samples one can also extrapolate to lower concentrations on curves which are simple and often linear.

Any atoms present in concentrations greater than a few per cent tend to affect the intensities of the spectrum lines from other atoms, so quantitative analysis becomes quite complicated when several major compounds of a mixture are varying. At concentrations of less than one per cent, however, most atoms do not have this mutual effect,

another reason why spectroscopic methods become especially useful at low concentrations.

The absolute precision of the spectrographic quantitative method is essentially constant at all concentrations and, depending on the care taken, should run between 20 per cent and 3 per cent. At the higher concentrations it is less precise than most chemical wet methods, and it improves relative to them as lower concentrations are approached. Roughly, the precision of the spectrographic method is equal to that of chemical wet methods between .5 and .01 per cent concentrations; below .01 per cent the spectrographic method usually becomes definitely more precise. Precision of 10 per cent at concentrations of one part in ten million is usually considered satisfactory.

Small quantities of material are sufficient for most quantitative spectrographic analyses as well as for qualitatives; and on occasion .1 milligram of sample will serve for a complete analysis, though ordinarily two- or three-milligram samples are desirable. A number of elements can be studied simultaneously, though this of course necessitates the preparation of proper comparison standards. In the case of food products these standards can often be made by adding weighed amounts of diluted solutions to the sample being analyzed.

A number of metallurgical problems arise in connection with food packaging. Many of these, such as analysis of tins used or lacquers for coating cans, and of steels that will not behave properly, can usually best be solved with the spectrograph. Often difficulties in rolling and coating of can stocks are due to quantities of impurities so small that they escape detection by ordinary wet methods.

Turning to the question of absorption spectrophotometry, useful in testing oils, liquors, lacquers, and other transparent materials, we find decreased qualitative accuracy but increased quantitative precision. Instead of narrow emission lines we now have broad absorption bands which may overlap and become inextricably tangled. Once a particular band has been established as arising from a given molecule, however, very precise determinations can be made of the amount of that molecule present in a solution.

The absorption method can be made even more sensitive than the emission method, a combination of a spectrograph and a microscope having been used to detect as little as 10^{-18} grams of metallic salts. A layer of material only a few atoms thick may so weaken a beam of light passing through it that it can thus be detected.

Several of the standardization methods for vitamin content of fish oils depend on absorption spectrophotometry. The potency of such material having been thoroughly standardized in terms of their effects

on rats or other experimental animals, the spectrograph is used as the working standardizer through its determination of absorption bands in the material.

Spectroscopic methods have already become important in food research; and as the importance of purity control is further stressed, their applications to such research should greatly increase.

AIR-CONDITIONING FOR FOOD PLANTS

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During these days of mass production and keen competition it is essential that the manufacturer of a food product, when he has once satisfied the taste of the public, be able to produce the article always the same not only in appearance but in taste and quality or he will soon lose his fickle customers. The manufacturer is also interested in keeping his costs of handling at a minimum, with a minimum of waste owing to spoilage.

While every food product is more or less a special problem, there are many problems in common that can be solved or improved by the use of conditioned air. Air-conditioning finds its greatest application in plants where foodstuffs are in contact with air during the manufacturing process. In the various food industries air-conditioning is applied for one or more of several reasons:

1. To control the temperature of the product while in storage or during the various stages of manufacture to prevent spoilage or loss of desirable qualities, such as appearance, taste, plasticity, etc.

2. Since most foodstuffs are hygroscopic and hence take in or give off moisture until in equilibrium with the surrounding air, it is important to control the moisture content in order either to maintain as nearly as possible the original moisture to prevent loss of weight, or to dry the product during the various stages of manufacture, as required.

3. To clean the air coming in contact with the product.

4. To produce a comfortable and healthful atmosphere in the factory for the benefit of employees.

5. In the retail trade to produce a comfortable atmosphere for the benefit of customers.

The heat and moisture content of the foodstuff, or the enclosure where it is processed, is seldom constant but in a continual state of flux. Where air-conditioning can be applied, it is the function of the air to remove or supply heat and moisture at the proper rate to maintain temperature and moisture content at an optimum.

By far the greatest number of problems involve the removal of heat and maintenance or removal of moisture, hence subsequent remarks will be confined to this type of problem.

The rate at which sensible heat and moisture, with its associated latent heat, are removed is dependent upon the temperature, humidity, and motion of the air coming in contact with the product. Since the air must rise in temperature to pick up sensible heat and rise in moisture content to pick up moisture and latent heat, the permissible rise in temperature or change in moisture content, i.e., absolute humidity, whichever is the controlling factor, then determines the volume of air which must be supplied to an enclosure. This volume, together with the volume of the space to be conditioned and the method for distributing air in the space, determines the air motion which will prevail. Careful design of the distribution system is essential to control the air motion over the product at the proper rate.

An air-conditioning system is only as good as its distribution system, and obtaining good distribution is one of the most difficult problems of the engineer. His aim is to insure that the temperature, humidity, and air motion in every part of the enclosure shall be the same within the permissible limits of variation.

Food technologists and engineers have, through their researches, determined the optimum temperatures, humidities, and air motions for storing and processing many foodstuffs. Unfortunately, the optimum conditions are difficult and sometimes impractical of accomplishment from an engineering point of view, and a compromise must be made in commercial practice. With meat in cold storage, for example, the loss of weight at 0°C. (32°F.) is practically three times as great at 85 per cent relative humidity as at 95 per cent relative humidity and approximately doubled at 10 feet per minute air movement as compared with still air. Loss of weight must be checked, not only for economic reasons, but on account of loss of color, flavor, and tenderness at the surface. On the other hand, for high humidities, above 90 or 95 per cent spoilage owing to bacterial action is apt to predominate because of moisture precipitating on the meat when fluctuations of temperature in the room bring the meat below the dew-point temperature of the air coming in contact with it. Below 90 per cent relative humidity and fairly constant temperature the spoilage is predominantly due to mold. For relative humidities below 85 per cent serious loss of weight occurs. As a compromise, commercial practice generally accepts 85 to 90 per cent as the optimum humidity range.

The possible limits of variation in temperature and humidity have an important bearing on the design and control of an air-conditioning system which can best be shown by one or two illustrations.

First, let us assume a room to be cooled and dehumidified where no fresh air is to be supplied but the same air is to be recirculated.

A (Fig. 1) represents a cooling surface usually cooled by a refrigerant at a temperature T_R and with a surface temperature in contact with the air (2) (Figs. 1 and 2) below the dew-point temperature of the air in the room. B (Fig. 1) represents a fan to draw air over the cooling surface and create a positive air circulation in the room; (1) represents the condition of the air as it returns to the conditioning unit. The slope of the line 1-2 is determined by the ratio of sensible heat to moisture pick-up of the air in passing through the room.

Part of the air at condition (1) comes in actual contact with the cold coil surface and is cooled and dehumidified to condition (2);

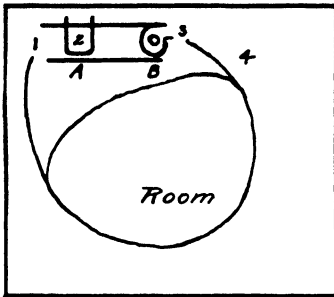


FIG. 1

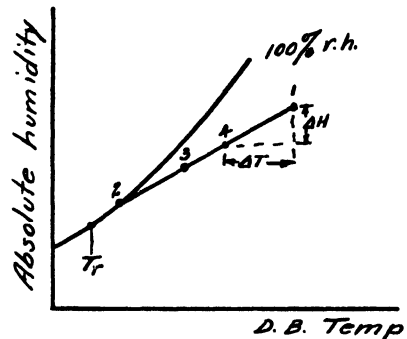


FIG. 2

the remaining part does not come in contact and by-passes the surface at its original condition, with the result that on leaving the fan at condition (3) it is a mixture of air at conditions (1) and (2). The air on leaving the fan at condition (3), owing to aspirating action, causes a diffusion of air at condition (3) with some room air at condition (1) to produce a mixture at condition (4) before coming in contact with the products in the room. In the occupied portion of the room there will therefore be a variation in temperature ΔT (4) to (1) and a variation in absolute humidity ΔH (4) to (1). Proper engineering design involves the proper choice and control of the temperatures and amounts of air circulated, so that condition (1) ΔT and ΔH and air motions will be within the desired limits.

In most air-conditioning problems it is necessary to take in some fresh air from outside for two purposes: (1) to supply fresh air for ventilation and (2) to create a positive pressure within the enclosure in order to cause a flow of air outward through cracks around windows and doors, thereby preventing as much as possible infiltration of unconditioned dust-laden air to affect conditions in the conditioned space. This affects the mechanism of cooling and dehumidification

so that in the occupied portion there will be a rise in temperature ΔT and an increase in absolute humidity ΔH . The slope of the line (1) to (3) is determined by the ratio of sensible heat to moisture pick-up in the room.

It frequently happens that the slopes of the lines (6) to (2) and (1) to (3), dependent upon different factors, are such that they will not intersect at condition (3). This is usually the case when the condition in the room must be maintained below approximately 50 per cent relative humidity. Within limits, this can be overcome by placing a heater between the cooler and fan, i.e., between A and B (Fig. 3). The result is illustrated (Fig. 5) where the path (7) to (3) represents sensible heat added by the heater necessary to bring point (3) on the line through (1) and (4), the slope of which was determined by the ratio of sensible heat to moisture pick-up in the room.

VITAMIN C CONTENT OF VEGETABLES

VIII. FROZEN PEAS¹

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Green peas have long been known as a good source of vitamin C. In early work in which no particular consideration was made of variety, maturity, sieve size, and freshness, the ascorbic acid (vitamin C) content of shelled peas reported has varied from .25² milligram per gram, found by Eddy, Kohman, and Carlsson (1926), to .14 milligram per gram, noted by McHenry and Graham (1935).

More recent work by Mack, Tressler, and King (1936), in which the above factors have been taken into consideration, has shown that they have a marked influence on the vitamin C content of peas. Freshly harvested green peas were found to vary from .12 to .40 milligram of ascorbic acid per gram.

Only a few workers have reported on the vitamin C content of frozen peas. Fellers and Stepat (1935) found that commercially frozen peas contain about 20 per cent less ascorbic acid than the freshly harvested vegetable. Further, they concluded that frozen peas are superior in vitamin C content to many of the shipped-in peas which are sold on the retail markets.

Since Fenton, Tressler, and King (1936) have shown that freezing peas does not cause an appreciable loss of ascorbic acid, it is evident that washing, blanching, cooling, and other commercial operations incidental to preparing peas for freezing are responsible for this loss. Recent work by Tressler, Mack, and Jenkins (1937) has shown that blanching lima beans in boiling water causes a loss of ascorbic acid varying from 19 to 40 per cent, depending upon the length of blanching period employed.

In view of these findings, a study was made under commercial conditions of the relative importance of different plant operations on the loss of ascorbic acid from peas during preparation for freezing.

¹ Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 211, July 27, 1937.

² Calculated on the assumption that .5 mg. of ascorbic acid per day is required for protection of a guinea pig from scurvy.

EXPERIMENTAL METHODS

The peas (Thomas Laxton and Alderman's Telephone varieties) used in this work were obtained from the same growers at Albion, New York, so as to avoid soil and climatic variations. These varieties were found to be similar in their ascorbic acid content (.24 mg. per gm.) when they were freshly picked and graded to the same sieve size, No. 5, at approximately the same stage of maturity.

According to commercial practice in preparing and freezing peas at the Snider Packing Corporation plant, they are vined, washed,

TABLE 1
Vitamin-C Content of Peas During Preparation for Freezing

Treatment	Total solids	Ascorbic acid content	
		Fresh basis	Dry basis
	<i>pct.</i>	<i>mg. per gm</i>	<i>mg. per gm.</i>
Freshly vined peas.....	24.1	.23	.96
After washing.....	23.1	.23	1.00
After blanching.....	24.0	.21	.88
Before shaker spray.....	24.8	.22	.89
Before quality separator.....	25.1	.21	.84
After quality separator.....	23.9	.18	.75
Before picking belt.....	23.3	.16	.69
After picking belt.....	22.4	.16	.71
Before filling.....	22.9	.16	.70
After packaging.....	21.3	.16	.75

blanched in boiling water, cooled in running water, packaged in cellophane-lined cartons and frozen in a Birdseye multiplate froster.

Controlled experiments were run in conjunction with the commercial pack so as to make direct comparisons for differences noted in loss of ascorbic acid at various stages in the process.

The Bessey and King (1933) titration method as modified by Mack and Tressler (1937) was used. A solution containing eight per cent of trichloroacetic acid and two per cent of metaphosphoric acid was used in the extraction process. One hundred milliliters of this solution extracts the ascorbic acid completely from 20 grams of peas and prevents any appreciable oxidation of the vitamin during analysis.

All frozen samples when analyzed for vitamin C were weighed at $-18^{\circ}\text{C}.$ ($-0.4^{\circ}\text{F}.$) so as to avoid errors in weight owing to condensation of moisture during this operation.

LOSS OF VITAMIN C DURING COMMERCIAL PROCESSING
FOR FREEZING

Peas for freezing are usually vined and handled as expeditiously as possible in the packing plant. At most, only a few hours elapse

from the time the peas are vined until they are frozen. At the time of this study peas of the Alderman's Telephone variety were being used. Samples were taken at different stages in the processing line and the ascorbic acid content determined immediately (Table 1).

It will be seen from these data that the most rapid loss in ascorbic acid content occurs after blanching rather than before. Blanching in itself resulted in a 10 per cent loss. With no delays in the processing line, the total loss of vitamin C was approximately 30 per cent.

EFFECT OF HOLDING PEAS PRIOR TO VINING

Three pecks of peas were picked from a truck load of freshly cut vines (Thomas Laxton variety) immediately on arrival at the freezing

TABLE 2
Effect on Ascorbic Acid Content of Holding Peas Before Vining

Treatment	Holding time	Temper- ature	Ascorbic acid content	
			Fresh basis	Dry basis
	<i>hours</i>	<i>°C.</i>	<i>mg. per gm.</i>	<i>mg. per gm.</i>
Vines held on platform.....	025	1.13
Vines held on platform.....	18	17-24	.24	1.08
Pods held in control chamber.....	20	6	.24	1.06
Pods held in control chamber.....	21	21	.25	1.09
Pods held in control chamber.....	18	32	.22	0.95

plant. A peck of these peas (in pods) was held 18 hours at each of the following temperatures: 6, 21, and 32°C. (42.8, 69.8, and 89.6°F.). The remainder of the load was spread out on the receiving platform for 18 hours. The maximum temperature during this period was 24°C. (75.2°F.), the minimum 17°C. (62.6°F.).

The peas in pods held at 32°C. lost approximately 10 per cent of their ascorbic acid content. At the two lower temperatures, however, no loss of vitamin C was noted, nor was there any appreciable loss of this vitamin from peas left on the vines (Table 2).

EFFECT OF VINING

Sometimes peas are vined faster than they can be blanched. As a result, oxidation of ascorbic acid may occur. In order to find out if this is the case, unwashed peas were selected immediately after vining and stored at 4 and 27°C. (39.2 and 80.6°F.) in perforated buckets for different periods of time (Table 3).

No apparent loss in ascorbic acid was noted in nine hours at 4°C.; whereas, in three hours at 27°C. a loss of 14 per cent of the original ascorbic acid occurred. In the regular commercial process of vining

and handling peas without delays, no appreciable loss of vitamin C occurred up until the time they were blanched.

EFFECT OF LENGTH OF BLANCHING PERIOD

Blanching, when properly conducted, inhibits the activity of the enzymes responsible for deleterious changes in color, aroma, and flavor during freezing, cold storage, and thawing. The inactivation of

TABLE 3

Loss of Ascorbic Acid From Vined Peas Held for Various Periods of Time

Holding time	Temperature	Ascorbic acid content	
		Fresh basis	Dry basis
hours	°C.	mg. per gm.	mg. per gm.
0	4	.28	1.20
3	4	.26	1.14
9½	4	.26	1.14
3	27	.24	1.02

catalase is commonly used as a criterion for determining an adequate blanching period. Catalase and ascorbic acid oxidase have been shown by Kertesz, Dearborn, and Mack (1936) to be inactivated by heat at the same rate. This would seem to indicate that loss of ascorbic acid from blanched peas because of enzymic oxidation is negligible. In

TABLE 4

*Effect of Length of Blanch¹ in Water at 93°C.(199.4°F.)
on Ascorbic Acid Content of Peas*

Time	Total solids	Ascorbic acid content	
		Fresh basis	Dry basis
sec.	pct.	mg. per gm.	mg. per gm.
0	22.5	.25	1.11
60	22.2	.21	.95
85	23.1	.20	.87
128	21.8	.17	.78
153	21.8	.16	.73

¹ A Scott blancher was used.

this work a Scott pipe-type blancher was employed in which the period of blanching could be increased at 20-second intervals. Immediately after being blanched, the peas were quickly cooled by immersing them in running water at 21°C. (69.8°F.), drained, and analyzed for ascorbic acid and catalase. None of the blanched peas was found to contain catalase when examined by the method described by Tressler and Evers (1936).

During blanching a portion of the ascorbic acid is lost (Table 4); this loss becomes greater as the blanching period is prolonged from 60 seconds to 153 seconds in water at 93°C.(199.4°F.).

EFFECT OF HOLDING BLANCHED PEAS PRIOR TO FREEZING

When the freezing machines are filled to capacity, peas may be held in packages on aluminum trays in a holding room at -1°C .

TABLE 5
*Ascorbic Acid Content of Peas Blanched in Various Ways,
Frozen, and Stored for Five Months*

Treatment	Time	Temperature	Content of ascorbic acid		Presence of catalase
			Fresh basis	Dry basis	
	sec.	°C.	mg per gm.	mg per gm.	
Steam blancher.....	0	0	.24	1.13	
	60	100	.18	.78	Negative
	80	100	.17	.74	Negative
	100	100	.16	.77	Negative
	120	100	.16	.74	Negative
	180	100	.16	.75	Negative
Sectt blancher.....	0	0	.25	1.10	
	60	82	.21	.90	Trace
	130	82	.21	.90	Negative
	156	82	.18	.79	Negative
Scott blancher.....	0	0	.25	1.04	
	60	88	.20	.88	Trace
	90	88	.20	.90	Negative
	122	88	.17	.76	Negative
	154	88	.16	.73	Negative
Scott blancher.....	0	0	.25	1.10	
	60	93	.21	.95	Negative
	128	93	.17	.78	Negative
	153	93	.16	.73	Negative
Berlin-Chapman blancher.....	0	0	.25	1.04	
	40	100	.18	.78	Negative
	60	100	.17	.77	Negative

(30.2°F.) for a few hours until they can be frozen. It seemed advisable, therefore, to determine whether or not there is any loss in ascorbic acid during such a holding period.

No significant changes were noted in the vitamin C content of peas held in packages at -1°C . for as long as 10 hours.

EFFECT OF THAWING ON VITAMIN C CONTENT

Fellers and Stepat (1935) found that peas, defrosted for two to six hours, retained only .04 milligram of ascorbic acid, a loss of nearly 70 per cent of the amount present in the frozen vegetable.

Samples of peas used in our study were previously blanched in boiling water for 40 and 60 seconds respectively and held in storage at -40°C . (-40°F .) for 11 months. In this work the frozen peas were weighed out at -18°C . (-0.4°F .), the packages were resealed and placed before a fan at 24°C . (75.2°F .); another sample was held at -4°C . (24.8°F .).

Thawing was not observed to cause much loss of ascorbic acid content of peas. This checks the work of Fenton and Tressler (1937) who similarly found little or no loss of vitamin C during thawing of peas.

RETENTION OF ASCORBIC ACID DURING STORAGE

Storage tests on peas were conducted for the purpose of determining the effect of methods and type of blancher employed on the retention of ascorbic acid.

This work shows that peas adequately blanched, frozen, and held in storage at -18°C . for five months maintained the same ascorbic acid content, .16 milligram per gram; whereas, unblanched peas having the active enzymes present and stored as above showed a decided loss in ascorbic acid (.23 to .15 mg. per gm.). Similarly, peas blanched in water at 93°C . (199.4°F .) for 60 seconds and held for seven weeks at -4°C . lost practically all of the ascorbic acid present (.21 to .04 mg. per gm.).

A definite relation (Table 5) exists between the retention of ascorbic acid and the time and temperature of the blanching period. The greatest retention of ascorbic acid was obtained by the following blanching procedures: in water at 82°C . (179.6°F .) for 130 seconds; in water at 88°C . (190.4°F .) for 90 seconds; in water at 93°C . (199.4°F .) for 60 seconds; in water at 100°C . (212°F .) for 40 seconds; and in steam at 100°C . for 60 seconds (Fig. 1).

The results indicate that peas blanched 60 seconds in water at 93°C . had been given the optimum blanch for conserving the greatest amount of vitamin C.

SUMMARY AND CONCLUSIONS

Studies of the changes in ascorbic acid content of peas during the commercial preparation for freezing indicated a loss of 30 per cent of the total amount present. A loss of 10 per cent occurred during the process of blanching. The most rapid loss of ascorbic acid was

found to occur during the cooling and washing operations subsequent to blanching.

The ascorbic acid content of freshly vined peas decreased during blanching, the loss becoming greater as the period of blanching in boiling water was increased. In a steam blanch, however, the rate of loss became negligible after the first 60 seconds.

It was found that the minimum loss of vitamin C occurred when the blanching period used was just sufficient to inactivate ascorbic

ASCORBIC ACID CONTENT OF PEAS BLANCHED IN VARIOUS WAYS

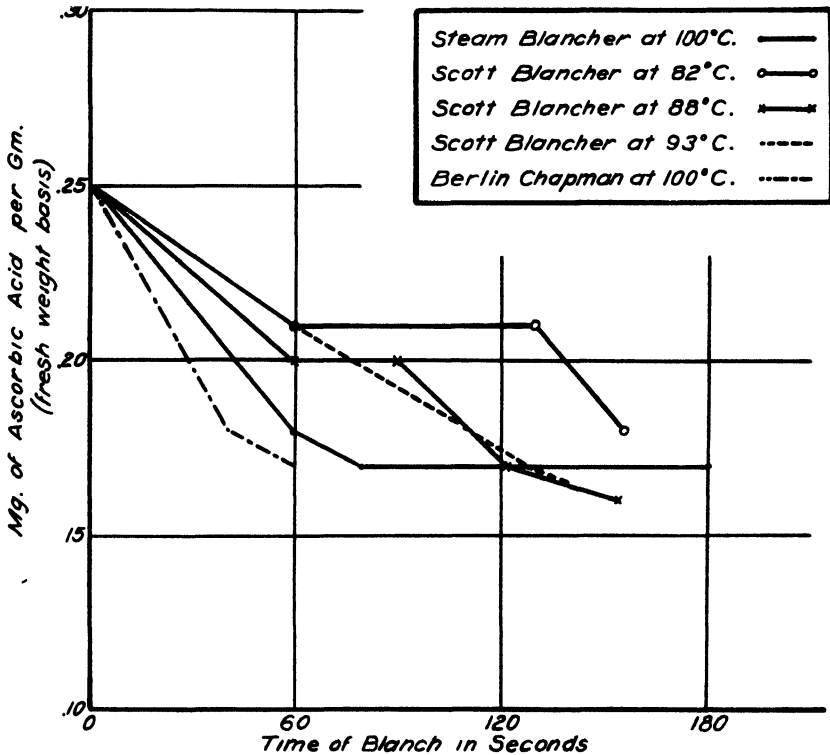


FIG. 1. Ascorbic acid determinations made on the frozen samples after five months' storage at $-18^{\circ}\text{C}.$ ($-0.4^{\circ}\text{F}.$).

acid oxidase, and the peas were then cooled and frozen immediately after blanching.

When peas were held at $-1^{\circ}\text{C}.$ ($30.2^{\circ}\text{F}.$) for 10 hours subsequent to packaging, no appreciable loss of ascorbic acid occurred.

Thawing peas at room temperature for 24 hours in sealed, moisture-proof packages resulted in no appreciable loss of vitamin C.

ACKNOWLEDGMENT

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APPLICATION OF SCIENTIFIC CONTROL IN THE BOTTLING INDUSTRY

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Our American practice of consuming refrigerated beverages undoubtedly serves to distinguish us, with respect to beverage habits, from most other peoples. The distinctive American beverage is neither alcoholic nor can it be served hot. It consists of flavored, non-alcoholic sugar syrups acidified with edible acids dissolved in water containing carbon dioxide gas, and it must be served cold.

Every phase of the carbonated-beverage industry is intimately associated with problems of refrigeration. In the older methods of carbonation, which are still employed in the smaller plants, the water, without precooling, is carbonated under high pressure and placed in bottles which must be closed almost instantaneously in order to prevent loss of the gas. This procedure results in the incorporation of relatively large quantities of air. This is undesirable both from the standpoint of keeping quality and retention of the carbon dioxide gas when the beverage is served. The newer and by far the better practice takes advantage of the considerably greater solubility of carbon dioxide gas in water at low temperatures. The water is refrigerated before carbonation, which can now be accomplished at very much lower pressures; and crowning may be carried out leisurely, for the cold water may be depended upon to retain the desired quantities of carbon dioxide, and the detrimentally excessive quantities of air, which are so characteristic of the high-pressure process, are avoided. Bacterial and particularly yeast spoilage are the most serious causes of beverage deterioration. Maintaining the beverage cool during storage markedly reduces losses from these agencies. Finally, the beverage must be served cold. Thus, in all phases of the carbonated-beverage industry—production, preservation, and consumption—refrigeration plays a prominent role. In fact, it is through the development and emphasis on refrigeration that the carbonated-beverage industry, as it is known today, is made possible.

NATURE OF CARBONATED BEVERAGES

The problems of scientific control may perhaps be most readily appreciated from a consideration of the nature and characteristics of

¹ American Bottlers Carbonated Beverages.

carbonated beverages and the fact that the industry comprises numerous small plants which are not in a position to employ trained technical men.

Carbonated beverages are compounded from flavored sugar syrups and fruit, or other edible acids; mixed with properly carbonated water; and packaged in clean, attractive, glass containers. The flavors and acids generally present no serious problems because these constituents are carefully standardized before reaching the bottling plant and, except for the true-fruit concentrates, are not likely to be a source of beverage spoilage. Carbonation, water treatment, syrup preparation, and bottle cleaning are thus the paramount problems of the bottled-beverage industry; these items will be briefly considered with respect to a few control procedures which have been developed.

The presence of carbon dioxide is the distinguishing characteristic of carbonated beverages. Its function is two-fold: first, it is responsible for the sparkle and the characteristic stimulating and refreshing properties and second, carbon dioxide aids materially in preservation of the beverage. It is customary to express the concentration of carbonic acid in terms of volume of the gas per unit volume of beverage. The quantity of gas employed must be varied for different beverages; for example, orange drinks generally contain one and one-half to two volumes of gas, while gingerales are prepared with three and one-half to four volumes of carbon dioxide.

It is important to maintain the carbonic acid concentration of a given beverage relatively constant. Nevertheless, field surveys have disclosed marked variations in the carbon dioxide content of what were presumably similar products produced by different plants; and, in some instances different bottles from the same plant have shown wide variations. These range from less than 10 per cent in a plant with proper and adequate control to 400 per cent in another plant which did not take advantage of adequate technical control.

To maintain a desired carbonation, the bottler depends on properly refrigerated water, pressure gauges on the carbonator and filler, pressure gauge for testing the final product, and proper closure.

In order to obtain the desired concentration of gas, the water must be maintained at a sufficiently low temperature, preferably 2.2 to 4.4°C. (36 to 40°F.), and the carbon dioxide should be applied at a constant predetermined pressure. Experience shows that low water temperatures are successfully maintained, but that unwarranted faith is frequently put on the dependability of pressure gauges at carbonator and filler, and testing of the final product is not adequately appreciated. The gauges are being continually subjected to wear and

tear with the inevitable result that they are occasionally in error. The gauges of the carbonator and filler should be looked upon primarily as guides and not as absolute measures of final carbonation of the product. The only way to determine the latter, obviously, is to ascertain the actual volume of gas in a certain proportion of the finished product.

Leaky crowns and inadequate closures are another source of carbonation variation. Submerging the filled bottles in water will generally serve to detect rapid leaks, and testing stored samples of beverages for carbon dioxide pressure will serve to detect slow leaks if this defect is at all common in a plant. A simple control procedure consists of storing a half dozen or more bottles from each batch and determining gas pressure from time to time over a period of one month.

The various pressure gauges must themselves be compared with a standard gauge from time to time to detect errors and defects. In view of the small size of individual plants, the national association of the carbonated-beverage industry (American Bottlers of Carbonated Beverages) has made provision to serve the bottler in this and other technical control procedures.

UNIFORMITY OF TASTE PRESENTS PROBLEMS

Since the appeal of carbonated beverages is to the taste of the consumer, it is essential that a given product should have uniformity of taste, wherever the beverage may be obtained. Even when the concentrated, flavored syrup is produced at a centrally located plant and distributed to bottling plants throughout the country, the finished beverages in various localities have been quite different in taste. Under the conditions stipulated the primary variable is the carbonated water. In view of the fact that the acids employed in beverages are a significant factor in taste, and that the differences in alkalinity of waters in different sections of the country necessarily are quite great, attention has been focused particularly on the neutralizing effects of water alkalinity on beverage acidity and consequently flavor. Recently there has been developed in the industry a tendency to treat water to remove the bicarbonate alkalinity with a view to producing nationally distributed products which will have more nearly the same taste. One of the processes of alkalinity removal consists of adding lime or lime and alum to the water, mixing thoroughly, and then storing for a short time (30 minutes to an hour) to afford an opportunity for sedimentation; or continuous-flow sedimentation tanks may be used. The supernatant liquid is then passed through a sand or other filter. Storage tanks employed are frequently 200 to 500 gallons in capacity. A plant

which has not been accustomed to cooling the water—generally they are plants using well water with temperatures of 10 to 12.8°C. (50 to 55°F.)—and which installs this alkalinity removal equipment has introduced a storage period which formerly did not exist. An opportunity is thereby introduced for a rise of temperature of the water which, in some instances, has turned out to be quite appreciable; for example, during periods of shut-down which are quite frequent in small installations. Where equipment is installed to remove or control alkalinity of ground waters, which are normally sufficiently cold, it becomes essential to provide simultaneously for adequate refrigeration and control of temperature of the water after treatment.

Suspended matters, tastes, and odors periodically encountered in water supplies constitute serious problems for the bottler, who is generally not qualified technically to cope effectively with them. Sand or stone filters, with or without the aid of coagulants, and generally supplemented with paper-disk filters, are very commonly employed for the removal of suspended matters, in most instances with considerable success. Charcoal filters or ozonation of water followed by filtration through activated carbon have been found very effective for the control of tastes and odors.

Deterioration of beverages is due primarily to yeasts and bacteria. The sources of these agents of spoilage are unsterile sugar syrups, contaminated pipe lines or utensils, and improperly washed bottles. Boiling sugar syrups and thoroughly cleansing, washing, and then sterilizing apparatus with hot water or chlorine solutions may be depended upon to control spoilage from these sources.

CLEANING AND STERILIZATION OF CONTAINERS

The problem of bottle washing and the economical production of adequately cleaned and sterilized containers is fundamental to the success of a beverage-bottling plant. In mechanical bottle washing, alkaline detergents consisting of caustic soda, sodium carbonate, trisodium phosphate, and, recently, sodium metasilicate in various proportions are generally employed. Studies by the research staff of the American Bottlers Carbonated Beverages have demonstrated that, on the basis of present knowledge, caustic soda must form the foundation for bottle-washing compounds. The milder alkalies, sodium carbonate, trisodium phosphate, and sodium metasilicate may be added for their auxiliary value, but they must not be present in sufficient quantity to interfere with lubrication properties of caustic soda on bottle-washing machinery. The A.B.C.B. recommends the following minimum re-

quirements as to composition, concentration, temperature, and period of exposure for mechanical bottle washing:

“Unclean bottles shall be exposed to a 3% alkali solution of which not less than 60% is caustic (sodium hydroxide), for a period of not less than 5 minutes at a temperature of not less than 130°F., or to an equivalent cleansing and sterilizing process.”

The majority of plants do not have trained personnel or laboratory facilities for standard chemical titrations. A rather simple tablet titration has been developed to enable the bottler to insure maintenance of the desired alkali concentration.

For determination of total alkalinity, a tablet consisting of potassium acid sulphate (KHSO_4) and the indicator tetra-brom-phenol-sulphon-phthalein (brom phenol blue) is employed. This indicator is violet-blue in alkaline and yellow in acid solutions. Each tablet contains sufficient acid to neutralize alkali equivalent to one per cent sodium hydroxide when added to 10 c.c. of solution. The tablets are so molded that half or quarter tablets may be easily employed. The number of tablets required to neutralize the alkali in 10 c.c. of bottle-washing solution thus becomes a direct measure of the alkalinity of the test solution expressed in terms of per cent of sodium hydroxide.

For determination of caustic alkalinity, which is considered the essential ingredient in bottle-washing compounds, the procedure is similar except that (1) a tablet of barium chloride (BaCl_2) is first added to the test solution to precipitate the carbonates and phosphates and most of the silicates which might be present; (2) the indicator employed is Trapaeolin O; and (3) the quantity of KHSO_4 present in each tablet is sufficient to neutralize caustic alkalinity equivalent to one-half per cent of sodium hydroxide. The color of the indicator is deep red-orange in alkaline and yellow in acid solution.

During the past year the traveling laboratory of the A.B.C.B. examined 106 samples of bottle-washing solutions. It was found that in 41 plants which were relying upon the hydrometers or other control procedures suggested by washing-compound supply houses only 18, or 44 per cent, of the washing solutions were up to the strength the bottler thought he had. In contrast to this, of 65 plants which were employing the tablet titration described above 60, or 92 per cent, were maintaining their bottle-washing solutions at the desired concentration.

DISCUSSION OF TABLET-TITRATION PROCESS

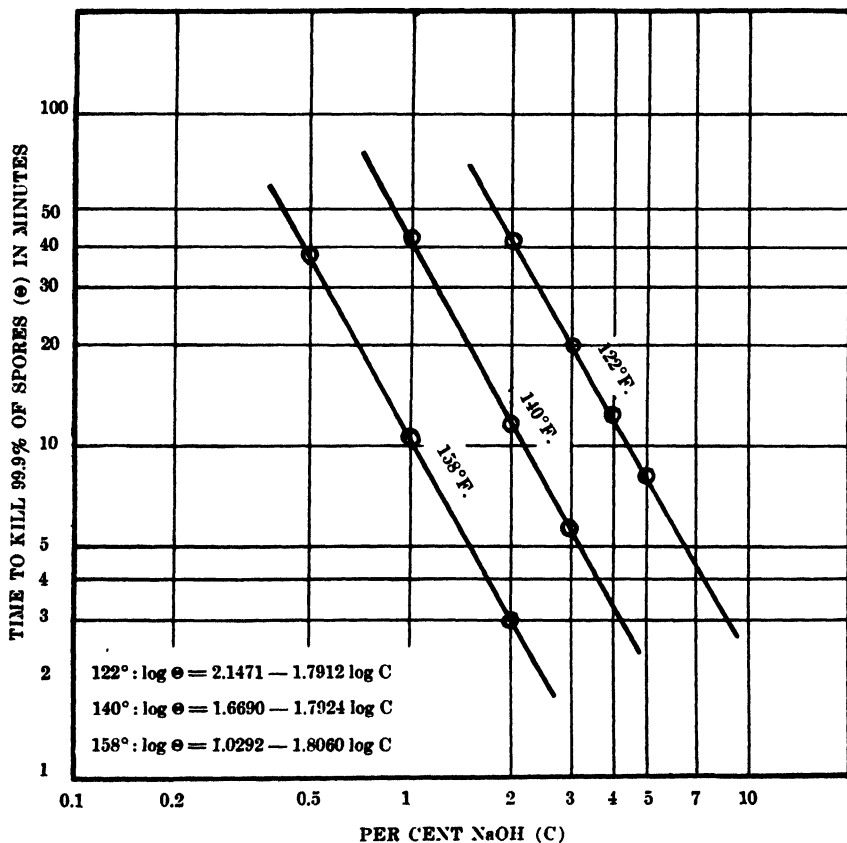
The tablet-titration process is obviously a convenient and dependable method; it is peculiarly well adapted to the conditions encoun-

tered in the carbonated-beverage industry and should be found equally applicable for control of bottle-washing solutions in other industries.

It is recognized that cleansing and sterilizing efficiencies are resultants of many factors, among which time of exposure, temperature, and concentration of the detergent are particularly important variables. It has further been ascertained that for sodium hydroxide at a constant

GERMICIDAL EFFICIENCY OF NaOH

B. metiens



$$\log e = K - a \log C - bt$$

FIG. 1.

temperature the logarithm of the concentration of caustic employed is inversely proportional to the logarithm of the killing time as shown in Fig. 1. Recently, C. R. Arnold, John M. Sharf, and the author,¹ of the research staff of the A.B.C.B., have suggested the following equa-

¹ J. Bacteriol. 34, 134, 1937.

tion for expressing the relationship between temperature, killing time, and concentration of sodium hydroxide:

$$\text{Log } \Theta = K - a \log C - bt$$

where

Θ = killing time in minutes

C = concentration of NaOH in per cent

t = temperature in degrees Fahrenheit

"K" is a constant associated with the resistance of the test organism or with other stipulated conditions, and "a" and "b" are constants associated with the detergent. For sodium hydroxide "a" and "b" have been found to be 1.7912 and 0.03129, respectively.

The New York City Health Department stipulates a minimum holding time of seven minutes, temperature of 65.6°C. (150°F.), and a concentration of two per cent of sodium hydroxide. For these conditions "K" = 6.07780. By use of the formula just described it is possible to develop tables of germicidal sterilizing equivalents so as to enable the bottler who is equipped to employ a higher temperature or a longer holding period than the minimum stipulated to reduce the concentration of alkali or in fact to vary any of these factors as may be deemed desirable or necessary for a local situation. Thus a degree of flexibility may be introduced in place of the present rigidity in setting bottle-washing standards. The use of this formula for determination of the germicidal equivalents, with respect to concentration of NaOH, temperature, and holding period for the conditions stipulated by the New York City Health Department for the sterilization of milk bottles is given here.

GERMICIDAL EQUIVALENTS

(Based on New York City specifications for milk bottles)

Killing Times for Designated Temperatures and Concentrations of NaOH

% NaOH	1.0	1.5	2.0	2.5	3.0
°F.	Time to kill (minutes)				
110	432.0	209.0	125.0	83.8	60.4
120	210.0	102.0	60.8	40.7	29.4
130	103.0	49.5	29.6	19.8	14.3
140	49.8	24.1	14.4	9.7	7.0
150	24.2	11.7	7.0	4.7	3.4
160	11.8	5.7	3.4	2.3	1.6
170	5.7	2.8	1.7	1.1	0.8
180	2.8	1.3	0.8	0.5	0.4

$\text{Log } \Theta = 6.07780 - 1.7912 \log C - 0.03129t$; Θ = killing time in minutes; C : NaOH; t = temperature in degrees Fahrenheit.

Further to assist the bottler to better control his product and operating processes, the A.B.C.B. has designed modified sugar hydrometers and measuring vessels and has issued a series of educational bulletins on various items concerned with manufacturing processes, particularly sanitation in the bottling plant. During the past year a motorized laboratory, completely equipped and specially adapted to cope with the chemical, bacteriological, and engineering problems peculiar to this industry, has been developed and sent out to aid individual plants and to gather pertinent information.

GAS STORAGE OF MEAT AND EGGS

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(Received for publication, November 12, 1937)

In the gas storage of animal products, such as meat and eggs, the aim is not only to control the metabolism and growth of living organisms—in this case invading bacteria and fungi, the foodstuff itself being “dead”—but to maintain as far as possible the initial condition of the foodstuff, notably its structure, appearance or color, and its taste. The success of gas storage in commercial practice depends upon how far these several conditions can be satisfied.

CHILLED BEEF

Chilled beef deteriorates because of the changes that take place near the surface of the meat, including slime and mold, rancidity in the fat, and loss of color or bloom. Given healthy, rested animals, properly cooled after slaughter, the changes that take place in the body of the meat are insignificant compared with these changes at the surface. If, however, changes at the surface can be arrested, autolytic changes remote from the surface will ultimately set a limit to the storage life of meat.

The bulk of the chilled beef imported into Great Britain comes from the River Plate, has an ocean journey of 18 to 25 days, and when sold in the shops is, on the average, 30 to 33 days from slaughter. Forty days from slaughter is about the limit that such meat can be kept in good condition unless the temperature of transport is lowered below the normal -2 to $-1.4^{\circ}\text{C}.$ (28.5 to $29.5^{\circ}\text{F}.$).

Carbon dioxide in any concentration retards the growth of the meat-attacking fungi, but in the region of $0^{\circ}\text{C}.$ ($32^{\circ}\text{F}.$) about 40 per cent is required to suppress growth completely, according to Moran, Smith, and Tomkins (1932), and Tomkins (1932). Likewise carbon dioxide slows down the growth of the bacteria responsible for slime on chilled beef, chiefly strains of *Achromobacter* and *Pseudomonas*, shown by Haines (1933). Rancidity in the fat of chilled beef is due chiefly to the breakdown of connective tissue framework by micro-organisms. At $0^{\circ}\text{C}.$ about 15 per cent of carbon dioxide will suppress completely the appearance of this form of rancidity over a period of 50 days at least, according to Lea (1933).

From the standpoint of preventing mold and slime on chilled beef a concentration of carbon dioxide of 40 per cent or higher is the ideal. With such concentrations, however, there is a marked increase

in the rate of formation of methaemoglobin in both muscle and fat so that the exposed muscle appears brownish and the fat bleached; in other words the bloom is destroyed. With low concentrations, however, (20 per cent or less) the loss of bloom owing to the specific action of the carbon dioxide over a storage period of the order of 50 days is negligible, as shown by Brooks (1933).

This, in brief, is the scientific analysis of the gas storage of chilled beef. It is in effect a compromise between the action of the gas in suppressing mold and slime and its effect on bloom. Clearly the factor controlling the storage life of meat is the weight of infection on the surface after slaughter and dressing. The smaller this is the longer the meat can be stored in a given concentration of carbon dioxide. In the ordinary way, however, beef stored at chilling temperatures in air containing 10 per cent carbon dioxide will remain free from mold

TABLE 1
Imports of Chilled Beef Carried in Carbon Dioxide Into Great Britain

Year	From New Zealand	From Australia
	cwt.	cwt.
1934.....	33,222	55,140
1935.....	110,697	231,857
1936.....	228,263	295,786
1937 ¹	176,989	171,062

¹ For the half year ending June 30

and slime for at least 60 to 70 days; this fact has been established both in the laboratory, by Moran and Smith (1932), and in commercial practice. The gas-storage method for beef has enabled Australia and New Zealand to export chilled beef to Great Britain. This trade is now well established and growing (Table 1).

On the average, Australian beef, when it is sold in England, is 50 to 60 days from slaughter (that from New Zealand is not so old). The bloom of this meat tends to be inferior to that from South America. This loss of bloom is most marked in the fat, particularly if the quarter is not well covered. Where the layer of fat is thin it is often bleached, the bleaching being due not only to the formation of methaemoglobin but also to actual oxidation, which we believe is due to catalytic oxidation. This latter, according to Lea (1937), is probably due to the catalytic action of an oxidase diffusing from the adjoining muscle. Theoretically the bloom should be improved if the surface of the meat could be dried more than at present. This postulates a lower relative humidity in the storage chamber, and this problem, including the purely engineering one of controlling the humidity in a space filled to capacity with chilled beef, is under investigation

at the present time. It is clear also that loss of bloom will be less the better the covering of fat on the quarter, i.e., the better the quality of the meat.

BACON

The storage life of chilled pork, mutton, and lamb can be extended with the aid of low concentrations of carbon dioxide. Bacon, however, is in a different category, the problem in this case being not only to prevent mold and slime but also oxidation of fat. The fat of bacon oxidizes rapidly, according to Lea (1931), and even at $-10^{\circ}\text{C}.$ ($14^{\circ}\text{F}.$) shows marked oxidative rancidity in a couple of months; at this temperature, incidentally, the oxidation of beef, mutton, and lamb is negligible at the end of 18 months. For this reason pure carbon dioxide is the ideal gaseous environment for the storage of bacon¹ and under such conditions can be stored free from slime and oxidative rancidity for a long period. Thus tank-cured bacon was stored at $0^{\circ}\text{C}.$ ($32^{\circ}\text{F}.$) for 18 weeks and was then indistinguishable from fresh bacon, as reported by Callow (1934). Storage at lower temperatures gives even better results, the storage life being longer while the flavor and texture are better preserved. Results are given of a semi-commercial experiment (Table 2) in which several sides of bacon, either tank-cured or dry-salted, were frozen at $-10^{\circ}\text{C}.$ ($14^{\circ}\text{F}.$) and stored at the same temperature in an atmosphere containing on the average 97 per cent carbon dioxide, the remainder being air; this was reported by Callow (1936).

A number of gas-stored sides which remained at the conclusion of the experiment were disposed of through normal commercial channels; the surplus of the air-stored bacon was inedible.

From a practical standpoint perhaps the main difficulty is that of obtaining an oxygen-free atmosphere. Storage up to 12 months is shown to be possible, however, even in the presence of .6 per cent of oxygen (Table 2), and with suitable precautions it seems likely that this concentration could be still further reduced.

EGGS

During the storage of eggs in air at ordinary or chilling temperatures, several changes take place, the more outstanding being (1) evaporation of water giving rise to a larger air chamber, (2) thinning of the thick white and shrinkage of the bag of thick white (enclosing the inner thin), (3) weakening of the yolk membrane, (4) a decrease in the viscosity of the yolk, and (5) the appearance of "storage taste" in the yolk. The problem of microbial attack during storage is simpli-

¹The formation of methaemoglobin in the exposed muscle of sides of bacon is not a factor of commercial importance.

TABLE 2
Gas Storage of Bacon at $-10^{\circ}\text{C}.$ ($14^{\circ}\text{F}.$) Compared With Storage in Air

Side	Type of bacon	Time of storage at $-10^{\circ}\text{C}.$ ($14^{\circ}\text{F}.$) mo.	Storage atmosphere	Measure of rancidity— peroxide content of back fat (c.c. 0.002 N, thiosulphate per gm.)	Flavor of cooked back fat
AB	Dry-salted	4	Carbon dioxide	4.9	No sign of rancidity.
AL	Dry-salted	4	Air	14.7	Definitely rancid.
BL	Dry-salted	7	Carbon dioxide	3.2	No sign of rancidity.
BR	Dry-salted	7	Air	16.1	Definitely rancid.
CL	Dry-salted	12	Carbon dioxide	3.6	Very slightly rancid.
CR	Dry-salted	12	Air	32.2	Excessively rancid.
DR	Tank-cured	4	Carbon dioxide	3.2	No sign of rancidity.
DL	Tank-cured	4	Air	29.2	Definitely rancid.
ER	Tank-cured	7	Carbon dioxide	6.5	No sign of rancidity.
EL	Tank-cured	7	Air	83.7	Excessively rancid.
FL	Tank-cured	12	Carbon dioxide	7.8	Very slightly rancid.
FR	Tank-cured	12	Air	75.2	Excessively rancid.

fied, according to Haines (1937), by the fact that in most eggs at laying the yolks and whites are sterile; on the other hand, the shell contains a heterogeneous flora and if the air in the store is too moist, mold grows on the shell and ultimately penetrates into the white and yolk. The passage of bacteria through the shell is much more difficult but the evidence is that it can occur and is facilitated if the egg has been robbed of its protective coating of mucin, probably by drastic washing.

The evaporation of water from eggs has been studied by Smith (1929). The most striking fact is that the evaporation at constant humidity is independent of the speed of the air passing over the eggs; there is also, as with most foodstuffs, a gradual decrease in the rate as evaporation proceeds.

The thinning and shrinkage of the thick white and the weakening of the yolk membrane are linked with the swelling and hydrolysis of the glucoprotein, mucin. Thinning is greater the more alkaline (higher) the pH, while the gross swelling of the bag of thick white behaves similarly with respect to pH. Our experiments have indicated that the quality of the white as a whole is best preserved if the pH is maintained at about 7.8, i.e., in air containing approximately two and one-half per cent of carbon dioxide. At the same time pH is not the only factor, and some shrinkage of the bag of thick white occurs whatever the conditions of storage. In this respect the white at laying is an unstable system, says Moran (1937).

The decrease in viscosity of the yolk is due to the passage of water from the white. Carbon dioxide at a concentration as low as two and one-half per cent retards this movement of water, Moran (1935), so that the quality of the yolks of eggs stored in carbon dioxide is superior to that of eggs stored in ordinary air.

The origin of "storage taste" is still obscure. Eggs will absorb stray odors but this is not the complete story. The first proof of this was obtained by Sharp and Stewart (1931) who found that the presence of carbon dioxide delays the appearance of "storage taste" in the yolk. This observation we have confirmed and it is an additional argument for the use of gas in the storage of eggs.

Normally the relative humidity surrounding the egg should not exceed about 85 per cent; otherwise mold develops on the shell. Carbon dioxide in any concentration retards this growth and therefore permits a higher relative humidity, but at 0°C. (32°F.) it must not be less than 60 per cent in order to suppress mold growth completely in a saturated atmosphere.

The gas storage of eggs is a particularly good example of the need for balancing of good and bad effects. Our experiments so far have

suggested two atmospheres of importance: (1) air containing two and one-half per cent carbon dioxide and the normal limit of relative humidity, e.g., about 85 per cent, (2) 60 to 100 per cent carbon dioxide and a saturated humidity. The first mixture gives a good-quality yolk and a firm thick white with a shrinkage only slightly less than after storage in air. With the second mixture there is no increase in the size of the air cell, the quality of the yolk is excellent, but the thick white is much shrunken so that the white as a whole is very watery.

In conclusion it should be noted that gas storage is not a new method of storage; it is simply a more elaborate form of cold storage involving, in addition to control of temperature and relative humidity, control of the composition of the gaseous atmosphere of the store. The use of this third factor of control makes storage more flexible; with many foodstuffs it will also prevent changes during storage which control of temperature and humidity alone cannot do.

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CALORIMETRIC INVESTIGATION OF FOODSTUFFS

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The Refrigerating section of the Food Industry group in Germany expressed the wish to redetermine the amount of heat to be abstracted from several foods during refrigeration in order to obtain reliable data for calculation of refrigerating costs, since many of the data hitherto published are highly contradictory. Accuracy of the measurements was to be at least five per cent, though it was considered desirable to obtain greater exactitude if possible.

METHOD OF INVESTIGATION

The large number of measurements to be carried out required a simple method. It was advisable to work with small samples (50 grams on the average), as larger quantities would have required an expenditure for apparatus out of all proportion. In the case of most of the ordinary ice calorimeters, interpolation of the course of temperature needs much calculation which becomes dispensable in the case of isotherm calorimeters, such as evaporation calorimeters. Such calorimeters have repeatedly been used to determine mean specific heat values between room temperature and the temperature of liquefied gases (air and hydrogen) corresponding to their normal boiling point, Eucken (1929). The substance to be tested was then placed directly in the boiling liquid and the quantity of vapor developed was measured. The drawback of such apparatus consists of inability to adjust more than one temperature. For that reason a new evaporation calorimeter was developed with the collaboration of Otto Schmitt, who carried out the majority of the measurements. The new calorimeter allowed the adjustment of temperature between 0 and -20°C . (32 and -4°F .) and guaranteed an accuracy of about one per cent.

In the new calorimeter, liquid sulfur dioxide was used since the heat of evaporation of this substance had been previously measured very carefully by the author. Liquid sulfur dioxide has saturation pressures at the refrigerating temperatures required that make the use of glass apparatus possible (Fig. 1). The calorimeter (at the extreme left in the figure) was surrounded by an ice-water bath in the Dewar vessel D_1 , in order to assure a constant heat transmission from outside. The silver-plated Dewar vessel D_2 , provided with a transparent band, contained the test room R, in which was placed the sample of foodstuff to be refrigerated. In this room a copper

vessel H was soldered in, by means of Wood's alloy, which, together with a methanol bath, secured a good conduction of heat into the surrounding liquid sulfur dioxide. The jacket containing the liquid sulfur dioxide was filled with sand S in its lower part to prevent delays in boiling, thus taking care of the proper saturation pressure above the liquid.

Let us follow the evaporated sulfur dioxide on its way from the calorimeter. First it passes along the mercury manometer M_1 , which serves as a safety valve, and flows to a Jena fritted glass filter FV. This filter is permeable for sulfur dioxide but not for mercury. It thus became possible to adjust and keep constant the vapor pressure

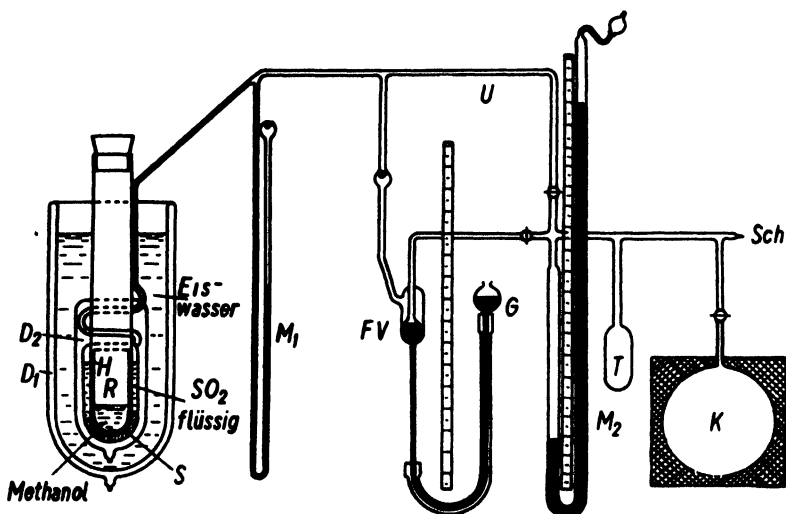


FIG. 1. Schematic representation of evaporation calorimeter used.

D_1 , D_2 —Dewar vessels; H—copper vessel; R—test room; S—sand; M_1 , M_2 —mercury manometers; FV—Jena fritted glass filter; G—mercury movable flask; U—by-pass; T—freezing pocket; K—glass vessel; Sch—melting place.

of the liquid sulfur dioxide in the calorimeter within $\frac{1}{2}$ mm. of mercury and to keep the evaporation temperature within corresponding limits. The filter was covered from below with mercury under a pressure which can be regulated by means of a movable flask G. Prior to each test this flask was adjusted to a pressure corresponding to the refrigerating temperature desired. The evaporating sulfur dioxide then pushed aside the mercury below the filter plate and passed into the part of the apparatus serving for quantity measurement. The quantity evaporated could be ascertained by measuring the pressure at manometer M_2 and by determining the temperature of the gas in the insulated vessel K, the volume of which was known. The freezing pocket T and the by-pass U were merely auxiliary for

introducing sulfur dioxide vapors from the vessel K into the calorimeter by condensation. The apparatus was filled with sulfur dioxide taken from a bottle and distilled; subsequently the apparatus was separated from the distillation plant at the melting place Sch.

The samples of foodstuff to be tested were put in small containers made of thin copper sheet, which filled the room R of the calorimeter, leaving a slight margin. Copper sheets, arranged in the form of a star inside the containers improved the heat exchange. Twelve hours before the beginning of a test the containers were placed in a bath of constant temperature which corresponded with the desired initial temperature of the foods to be refrigerated.

The measurements were carried out in the following way: After adjustment of the required cooling temperature a constant course of evaporation (course of evaporation analogous to course of temperature) was awaited. Then the foods were introduced into the calorimeter. About one and one-half hours elapsed before the original course of evaporation was reached again. Thus it was possible to calculate from the evaporated amount of sulfur dioxide the quantity of heat abstracted. Before the measurements with foodstuffs were made, of course, several check tests were made using a piece of copper of well-known specific heat. After the elimination of a few initial errors the calorimeter yielded results having an accuracy within one per cent.

Soon it became evident that two of the tested foodstuffs (butter and onions), when cooled below freezing point, could not yield their full heat content, for the results fluctuated far beyond the expected limits of error. This was found to be due to the fact that the temperature equilibrium (solid liquid) of the water component did not take place but that subcooling phenomena occurred.

An attempt was made, therefore, to force the equilibrium by means of ultra sound waves. The experimental procedure is shown schematically (Fig. 2) and it is adapted from a disposition which Vincent (1929) has proposed for tests with oscillating nickel bars. In the oscillating circle S of an ordinary reaction transmitter a coil was inserted and wound on the foodstuff container B made of nickel, in this case. By altering the capacity C the frequency of the oscillating circle could be so adjusted that the nickel container, under the influence of the high frequency magnetic field, was stimulated to mechanical longitudinal oscillations.

In the preliminary experiments, carried out by E. Boehm, the mechanical oscillations of the nickel container were demonstrated by means of an auxiliary circle (Fig. 2). A magnetic iron pin soldered onto the container B dipped into the coil SP of a detector circle; the coil was protected against induction by plate P. When the cylinder showed mechanical resonance, the microammeter μA indicated

a strong deflection. At the same time the grid current showed a marked fall at the instrument mA which outside the resonance sprang back again to its original value. In the course of these experiments it was observed that annealed nickel had a much broader and, for our experiments, a more favorable resonance curve.

In the calorimetric measurements the detector circle was dispensed with and the resonance was here merely ascertained at the grid current

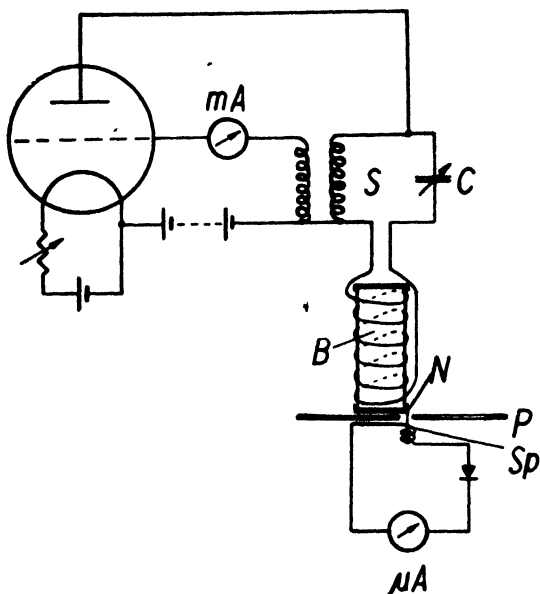


FIG. 2. The ultra sound transmitter.

S—oscillating circle; C—condenser; B—nickel container; N—iron pin; P—protecting plate; Sp—coil of the detector circle; mA—milliammeter; μ A—microammeter.

ammeter mA; the container, together with the coil, was surrounded by an insulating rubber jacket so as not to have to dispense with methanol, so important for the heat exchange in the calorimeter. The transmitter, after an apparently finished heat exchange, was switched on for about one minute in the experiments in question, whereupon an additional heat development in the calorimeter was usually observed. Generally it was not enough to switch on the transmitter once in order to freeze the highest possible amount of water in the foodstuff tested. Then the transmitter was switched on for a short time at certain intervals until no additional heat exchange was observable.

RESULTS OF EXPERIMENTS

The foodstuffs tested and the results obtained are indicated (Table 1). The temperature intervals and the nature of the goods to be

refrigerated were as prescribed by the Refrigerating section of the Food Industry group.

On account of the different composition of animals, measurements of meat were carried out separately for muscle flesh, fat, and bones. Subsequently the heat content of a whole animal could be calculated. As to muscle flesh, the results accord closely with the previous measurements by Heiss (1933). He found a frozen quantity of water of 81.5 per cent at $-8^{\circ}\text{C}.$ ($17.6^{\circ}\text{F}.$), whereas we found 80.8 per cent. In the case of haddock the frozen quantity of water was found to be

TABLE 1

Foods refrigerated	Cooling interval	Water content	Refrigeration duty required
	$^{\circ}\text{C}.$	pct.	kcal/kg. ³
Beef ¹	20 to -8	52.0	49.0
Pork ¹	20 to -8	54.0	50.0
Haddock.....	20 to -8	80.0	73.2
Cottage cheese (10% fat).....	20 to -5	78.5	74.3
White cabbage.....	20 to -1	92.5	20.0
Eggs.....	20 to -0.5	67.0	14.7
Onions ²	20 to -3	86.0	66.5
Unsalted butter ²	25 to -3	16.0	28.3
Unsalted butter ²	15 to -3	16.0	22.5
Unsalted butter ²	15 to -8	16.0	25.5
Salted butter (3% salt).....	25 to -3	16.0	18.6
Salted butter (3% salt).....	15 to -3	16.0	12.8
Salted butter (3% salt).....	15 to -8	16.0	19.2

¹ The above-mentioned figures are valid for average animals. The following fluctuations are possible:

Lean beef..... 52 kcal/kg. (18% bones; 21% fat; 61% muscle flesh)
 Normal beef..... 49 kcal/kg. (18% bones; 25% fat; 57% muscle flesh)
 Fat beef..... 45 kcal/kg. (18% bones; 38% fat; 49% muscle flesh)
 Lean pork..... 54 kcal/kg. (8% bones; 24% fat; 68% muscle flesh)
 Normal pork..... 50 kcal/kg. (8% bones; 30% fat; 62% muscle flesh)
 Fat pork..... 48 kcal/kg. (8% bones; 36% fat; 56% muscle flesh)

² Experiments carried out by the aid of the ultra sound transmitter.

³ Multiply by 1.8 to convert the values of this column into BTU/lb.

81.3 per cent in conformity with British investigations by Chipman and Langstroth (1927). In the case of cottage cheese a frozen quantity of water of 81.8 per cent was found. Eggs and cabbage did not freeze at the required refrigerating temperatures of $-0.5^{\circ}\text{C}.$ ($31^{\circ}\text{F}.$) and $-1^{\circ}\text{C}.$ ($30.2^{\circ}\text{F}.$) respectively; consequently the amount of refrigeration required is comparatively slight. The experiments hitherto mentioned were made without the aid of ultra sound waves. In the case of onions and butter, however, the results varied greatly in the first series of experiments, owing to differences caused by subcooling. The values given (Table 1) for these substances were then determined with the aid of the ultra sound transmitter. In the case of butter there is a remarkably large difference in the amount of heat

abstracted from salted and unsalted butter, but this may be understood when it is born in mind that the salt concentration in the salted butter amounts to roughly three moles per liter of water content.

The results gained until now provide, of course, only a small number of data. These do not yet suffice for the exact calculation of the amount of heat to be abstracted from foodstuffs in a wide range of temperature. As the composition of foodstuffs, especially as regards their water content, is subject to variations that greatly influence the amount of refrigeration required, the figures given here are only average values.

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CRISTALLISATION ET DESSICCATION DE CERTAINS PROTEIDES SOUS L'ACTION DU FROID

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I. Jusque dans ces dernières années on a considéré que l'état cristallin constituait le principal caractère différentiel entre les colloïdes et les cristalloïdes. Jacques Loeb, dans sa théorie des phénomènes colloïdaux, écrivait après Baudrimont, Thomas Graham, J. Duclaux, Auguste Lumière et tant d'autres : "on ne peut séparer les phénomènes de la vie des propriétés colloïdales et l'idée d'un organisme ou d'une matière vivante exclusivement ou principalement formée de cristalloïdes ou de matières douées de propriétés purement cristalloïdes est purement inconcevable."

Or, il apparaît de plus en plus nettement que les substances que l'on considère comme uniquement colloïdales peuvent, cependant, prendre l'état cristallisé. *La vie cesse à la limite de ces deux états continus de la matière.*

Sans parler de l'oxyhémoglobine dont l'extraordinaire facilité de cristallisation est bien connue de tous, nos recherches sur les protéines, exposées tout récemment en détail dans l'ouvrage¹ "La biochimie des protéines" établissent que notamment les trois protéines du sérum sanguin ont la propriété de prendre l'état cristallin au sens ou l'entendent les chimistes.

On sait qu'en substituant à la *méthode aux sels*, utilisée classiquement pendant près de 100 ans, pour la séparation des protéines, notre *méthode à l'acétone* aux basses températures, il a été possible d'isoler trois protéines différentes par leur composition chimique et leurs propriétés physiques : la sérum albumine et la sérum globuline déjà connues et la myxoprotéine nouvelle substance protéique nettement distincte.

Ces trois protéines, grâce à l'état très grand de pureté dans lequel elles sont obtenues par cette technique, sont susceptibles de cristalliser dans des conditions expérimentales déterminées.

Rappelons d'abord sous quelle forme elles sont isolées du sérum.

La "sérumglobuline" séparée, la première, par floculation au pH moyen 6.4 se présente sous l'aspect de petits floculats blancs, d'aspect grenu, se maintenant longtemps en suspension dans le milieu aqueux. Après deux lavages à l'eau distillée, par centrifugation, les floculats

¹ Librairie de J. B. Baillière, Paris 1937.

se gonflent peu à peu. Insolubles dans l'eau, ils se dispersent aisément dans les électrolytes forts (acides et alcalis).

La "myxoprotéine," isolée ensuite par plusieurs précipitations successives à froid en milieu acétonique, se sépare du milieu aqueux à l'état de gros flocons grisâtres qui s'agglomèrent rapidement et tombent au fond du récipient en une masse visqueuse, de teinte blonde, qui par synérèse expulse une liqueur aqueuse limpide. D'une solubilité extrêmement faible dans l'eau pure (moyenne 1 0/00), cette protéine se disperse facilement dans les électrolytes faibles (sels neutres alcalins à 10 0/00), mieux encore dans les électrolytes forts.

La "sérumalbumine" enfin restée en solution dans l'eau pure donne une liqueur, d'un blond champagne, d'une transparence parfaite. Elle ne flocule ni par les acides, ni par les bases, ni par les sels alcalins neutres sauf à concentration massive (par exemple: sulfate d'ammoniac à saturation).

Voici rapidement réunies les conditions de cristallisation de ces protéines.

La *sérumalbumine* obtenue en général avec une teneur en substance sèche de quatre pour cent, doit être amenée à une concentration voisine de 50 pour cent. On y parvient par deux procédés; (1) soit en ajoutant progressivement à la solution d'albumine, à la température du laboratoire, 1/3 environ de son volume d'alcool éthylique à 96 il se précipite alors une matière huileuse blonde (contenant 45 pour cent de poids sec) qui, refroidie dans la glace, se prend en une masse cristalline, fondant à la chaleur de la main, (2) soit par évaporation sous vide sulfurique jusqu'à élimination de 96 pour cent environ de l'eau.

Dans cette dernière technique, de beaucoup la plus sûre, la protéine se présente sous l'aspect d'une masse colloïdale, admirablement transparente, qu'il suffit d'étaler en couche mince et de refroidir aux environs de 13°C. (55.4°F.) par un mélange de glace et de sel pour la voir cristalliser rapidement en une substance blanche, d'aspect soyeux, à reflets moirés, formée de prismes serrés les uns contre les autres et mélangés de cristaux de glace provenant de la cristallisation de petites quantités d'eau.

Pour isoler ces cristaux d'albumine des cristaux de glace nous avons eu recours à la distillation de l'eau, en chambre froide à -5°C. (23°F.) sous ventilation. L'eau va se condenser sur le frigorigère, laissant seuls les cristaux de la protéine qui cependant ont changé de consistance; d'abord *mous*, s'écrasant sous la moindre pression, ils deviennent durs, *solides*, très friables.

La photographie jointe montre sur une crête cristalline, un gros prisme type.

Dans des conditions analogues, on a pu faire cristalliser diverses albumines, isolées des milieux biologiques (lait, blanc d'oeuf) ou de protoplasmas cellulaires (fibre musculaire striée, fibre lisse, cellule cancéreuse).

La *myxoprotéine* cristallise également en masse (cristallisation diffuse) au contact d'un mélange de glace et sel. La protéine perd rapidement sa consistance pâteuse et se transforme en une matière dure, cassante, de coloration blanc-grisâtre. La dessiccation se fait au froid comme pour l'albumine.

La cristallisation de la *sérumglobuline* n'a été obtenue qu'accidentellement et très rarement au cours de très nombreuses manipulations de dessiccation sous vide sulfurique, pendant les hivers froids



FIG. 1. Sérumalbumine cristallise: cristal sur une crête cristalline.

où la température du Laboratoire s'abaissait fortement. Tandis que dessechée à l'état amorphe, elle se présente sous forme de lames cornées, transparentes, très dures, au contraire, lorsqu'il y a eu cristallisation, la protéine est de coloration blanchâtre, très friable, agissant fortement sous la lumière polarisée. En général partielle, la cristallisation se produit à la fin de l'élimination de l'eau dont l'évaporation provoque vraisemblablement un notable abaissement de la température qui amorce la formation de dépôt cristallin.

Les accidents de cristallisation au cours de dessiccation sous vide sulfurique sont très fréquents dans le cas de la sérumalbumine et de la myxoprotéine. Tout dernièrement, la *globine* provenant de la

scission de l'oxyhémoglobine cristallisée de cheval a donné lieu à des constatations analogues.

L'ensemble de ces faits montre que non seulement les protéines mais encore certains protéides plus complexes sont capables de prendre l'état cristallin jusque là seulement réservé aux cristalloïdes lorsqu'on réalise les conditions expérimentales suivantes :

1.—*préparation à l'état pur* et par pureté il faut entendre non seulement l'élimination des électrolytes et non électrolytes divers mais aussi l'isolement de chaque substance protéique de toute autre.

2.—très forte *concentration* des solutions ou suspensions aqueuses des protéines amenant leurs molécules en contact intime.

3.—*abaissement de la température* au moment où ce contact est devenu convenable.

II. Les expériences indiquées plus haut en vue de séparer les cristaux de sérumalbumine des cristaux de glace qui les accompagnent en distillant l'eau de ces derniers, à basse température, nous a conduit à une technique nouvelle dans les Laboratoires, la *cryo-dessiccation*.

On sait depuis longtemps que l'eau, bien au-dessous de 0°C. (32°F.) possède une tension de vapeur qui permet de la condenser sur paroi plus froide, dans le cas particulier, sur frigorifère. On sait aussi qu'un stockage trop prolongé des denrées même congelées s'accompagne par évaporation d'une perte de poids qui peut être très importante.

La cryo-dessiccation des protéines cristallisées a été obtenue en chambre froide maintenue aux environs de -5°C. (23°F.) dans le frigorifique expérimental annexé au Laboratoire des Halles Centrales de Paris.

Dans les premiers essais le produit à dessécher étalé en couche mince dans des plaques de Pétri était déposé sur des claies métalliques. Plus tard on a eu recours à un tunnel métallique de 35 cm. de long, à grand axe perpendiculaire au frigorifère; un petit ventilateur aspirait l'air froid de la chambre à travers le tunnel contenant la matière à dessécher et le dirigeait sur le frigorifère où il se déshumidifiait. Dans ces conditions la dessiccation est beaucoup plus rapide.

Les expériences ont porté sur de nombreux extraits organiques, sur la sérumalbumine cristallisée mélangée de glace et, enfin sur l'oxyhémoglobine cristallisée de cheval.

Sur de très beaux cristaux d'oxyhémoglobine contenant 44 pour cent d'eau (étuve 103-104), 42 pour cent (vide sulfurique) on a suivi la marche de la dessiccation en fonction du temps: l'eau perdue a été de 14.2 pour cent dans les 24 premières heures, 7.3 après 12 heures et 2.4 (12 heures). Après quatre jours, la perte totale en eau s'élevait à 29.43 pour cent.

Les propriétés physiques de l'oxyhémoglobine ainsi deshydratée: coloration, solubilité sont très peu modifiées dans ces conditions.

Il semble que cette technique extrêmement douce pourrait être appelée à rendre de grands services pour la préparation de substances délicates: protéides divers, produits opothérapiques, etc. En même temps, elle appelle l'attention des frigoristes sur l'étude, encore quelque peu insuffisante, des pertes de poids des denrées soumises à l'entreposage frigorifique.

ESSAIS DE "QUICK FREEZING" APPLIQUE AUX GROSSES PIECES DE VIANDE

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Dans les sciences appliquées il est presque de règle que la théorie suive de loin l'expérimentation.

C'est ainsi que le *sharp freezing* qui remonte tout-à-fait au début de l'application du froid aux viandes n'a été étudié théoriquement qu'en 1916 par le professeur Plank dont les beaux travaux ont été continués ensuite par de nombreux auteurs Américains, Anglais, Canadiens, et Allemands.

Les essais de quick freezing ont suivi de près la réalisation du *sharp freezing*. Les premiers semblant bien devoir être attribués aux Américains: D. W. Davis (1869), et D. W. Davis et S. H. Davis (1871). On signale en 1870 les brevets de Howell (U. S.) des anglais Thew (1882), de Hesketh et Marcet (1889). Puis se succèdent les procédés bien connus d'Ottesen, Taylor, Henderson, Hirsh, Piqué, Goerz de Hervé, Petersen et Bridseye, Birdseye et Vogt, Zarotschenzeff, les uns préconisant l'action directe de l'agent frigorigène sur la denrée, les autres l'action indirecte (parois métalliques, sacs de toiles, membranes en caoutchouc, en cellophane, etc.).

Sans doute les conceptions théoriques du Professeur Plank purent être étendues à la congélation rapide, cependant il reste encore bien des recherches à faire notamment en ce qui concerne les denrées végétales. Jusque là on s'est contenté de dégager grossièrement les principaux avantages résultant du *quick freezing* et Tressler 1932 les a résumés dans les conclusions suivantes:

- "1. Les cristaux de la glace pendant la congélation rapide sont beaucoup plus petits et par conséquent causent moins de désordre dans les cellules.
- "2. La durée de congélation étant beaucoup plus courte, beaucoup moins de temps est laissé à la diffusion des sels et à la séparation de l'eau sous forme de glace.
- "3. Le rapide refroidissement au-dessous de la température favorable au développement des bactéries, moisissures, empêche l'altération des denrées pendant l'action du froid."

En ce qui concerne plus particulièrement la congélation des fruits et des produits végétaux, une congélation extrêmement rapide réduit au minimum les désordres des tissus, les phénomènes d'oxydation, les fermentations et le développement des microorganismes.

Le problème du *quick freezing* a été résolu pratiquement aux U.S.A. pour la très grande majorité des denrées périssables. Il serait

superflu de citer les statistiques de la *Général Foods Corporation* établissant la progression formidable des aliments conservés en paquets, à tel point que l'on a pu dire que cette industrie a causé une véritable révolution dans l'approvisionnement des grands centres urbains.

Toutefois la congélation rapide n'a pas été appliquée avec pleins succès aux produits présentés sous une grande épaisseur : carcasses de petis animaux de boucherie (veaux, porcs, moutons), quartiers de gros bétail. Elle aurait cependant un énorme intérêt pour les pays grands exportateurs de viandes frigorifiées (Argentine, Uruguay, Brésil, Australie, Nouvelle-Zélande, Sud-Afrique, etc.).

C'est faute d'une telle adaptation que ces pays ont du abandonner progressivement la préparation des viandes *frozen* pour celle des viandes *chilled*.

On aura une idée de cette évolution technique en consultant les statistiques par exemple de l'exportation Argentine des viandes frigorifiées de l'espèce bovine de 1914 à 1936 :

EXPORTATIONS TOTALES

<i>Année</i>	<i>Chilled</i>	<i>Frozen</i>
1914	40.690	328.278
1919	38.995	355.842
1921	2.480	398.251
1925	148.386	241.372
1927	466.669	236.420
1932	370.634	36.660
1934	349.644	31.584
1935	348.531	30.651
1936	357.472	39.650

Il a suffi de 25 années pour que le *chilled* ait remplacé presque exactement le *frozen*, et la disparition de ce dernier eut été totale si le continent n'avait continué à l'acheter pour des raisons de commodité et disons le aussi de pratique routinière.

Evidemment ce sont les inconvénients importants de la congélation *semi-lente* qui ont imposé ce changement si rapide, surtout de la part des consommateurs Anglais, les meilleurs experts en viandes. Mais ces inconvénients ne sont pas les seuls, d'autres reproches graves ont pu être faits aux viandes *frozen* : d'abord la qualité des animaux insuffisamment améliorée, puis les modifications ou altérations dues à la décongélation et surtout à la condensation de l'humidité atmosphérique entraînant poussières et microorganismes de l'air, enfin un stockage en général beaucoup trop prolongé entraînant des pertes sensibles de poids, un brunissement des viandes et toujours une notable rancidité des graisses, non seulement superficielles mais profondes atteignant parfois jusqu'à la moelle des os. Ce dernier facteur, rancidité, de dépréciation des viandes congelées est sans doute celui qui a le plus nuit à ces viandes.

L'orientation vers les viandes *chilled* a donc permis aux pays exportateurs de donner satisfaction à leurs acheteurs et de maintenir leur commerce. Elle leur coûte d'ailleurs de notables sacrifices notamment en ce qui concerne le fret nettement plus élevé, à tonnage égal, que celui de viandes *frozen*, sacrifices cependant compensés en partie par des meilleurs prix de vente. En outre, pour les transports à longue distance (Australie, Nouvelle-Zélande notamment) il a fallu recourir aux cales frigorifiques étanches au CO² (dr. T. Moran) et augmenter dans de grandes proportions la vitesse des bateaux frigorifiques, d'où des dépenses très considérables se répercutant sur le prix des viandes *chilled*.

Il ne semble pas douteux aujourd'hui que l'on puisse revenir dans une certaine mesure aux viandes congelées, à la condition de leur appliquer la technique du *quick freezing* avec ses avantages bien connus. C'est que d'une part, les progrès de l'élevage en Sud-Amérique, principalement, puis en Australie, Nouvelle-Zélande, et Sud-Afrique, ont été considérables amenant l'ancienne qualité continentale à la qualité *chilled*. D'autre part, sous l'action vigoureuse de l'Institut International du Froid, la sous-commission des viandes frigorifiées créée en son sein a mis à l'ordre du jour, à la section économique de la S.D.N. (League of Nations), l'obligation pour toutes les denrées conservées de porter le lieu et la date de préparation, obligation qui équivaut à un contrôle et par suite à une limitation de leur entreposage d'où atténuation très sensible des inconvénients des modifications ou altérations dues à celui-ci.

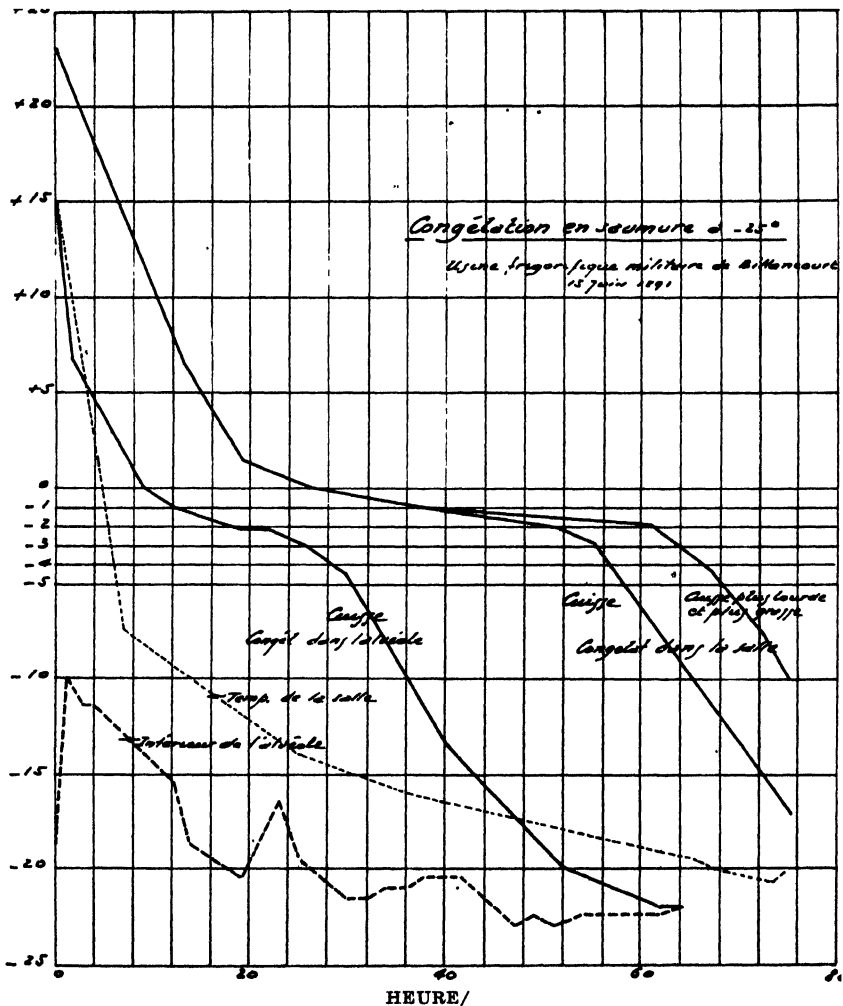
Des viandes congelées préparées dans ces conditions verraient les marchés mondiaux s'élargir notablement devant elles, car elles répondent tout de même à des besoins qui n'ont pas diminué bien au contraire. L'économie du fret, la possibilité d'une conservation plus longue leur donneraient des avantages considérables surtout pour les approvisionnements militaires (armée et marine).

Les premiers essais de congélation relativement rapide, en France, tout au moins, remontent aux environs de 1891. Ils ont été effectués à Billancourt (Seine) sous le contrôle d'une importante commission de l'Armée présidée par de Freycinet et conduits par Mr. A. Barrier, alors Ingénieur de la Marine, qui devait être plus tard notre prédécesseur à la Direction de l'Institut International du Froid. Mr. A. Barrier a bien voulu nous communiquer, parmi tant d'autres recherches sur la conservation des denrées par le froid, quelques uns des résultats les plus typiques obtenus à cette époque.

Utilisant de grands mouleaux à glace (alvéole), refroidis extérieurement par de la saumure à -26°C. (-14.8°F.), sans cesse en circulation, Mr. Barrier a congelé des quartiers de viande en 50 heures, avec

un assez court palier de 15 heures entre 0 et -4°C . (32 et 24.8°F .), comme le montre la courbe ci-dessous :

Tout récemment des essais beaucoup plus caractéristiques de *quick freezing* sur des grosses pièces ont été effectués, en France, aux Glacières d'Arras, par l'Ingénieur frigoriste Hoveman, d'après une



technique brevetée dans les principaux pays d'Europe et d'Amérique.

A la vérité les brevets Hoveman sont avant tout des brevets d'*application*, car les principes sur lesquels ils reposent avaient déjà été envisagés théoriquement, comme il a été dit, plus haut, puis précisés en 1933 par les recherches de l'Ingénieur Dr. R. Heiss, élève et collaborateur du Professeur Plank, à l'Institut Technique Frigorifique

de Carlsruhe. Dans un important travail, Rudolph Heiss écrit en effet :

“L’une des méthodes susceptibles d’éviter le changement de coloration de la viande sous l’action du froid, consiste à la congeler dans des enveloppes imperméables (procédé indirect de congélation rapide, grâce à une couche séparatrice entre la saumure et la viande). On est parvenu en congelant dans la saumure, à réaliser un produit absolument identique à la viande congelée dans l’air, sous le rapport des modifications de teintes. Son application pratique consisterait à introduire de gros morceaux de viande, par exemple des quartiers de viande de boeuf dans des sacs en caoutchouc. Pour arriver à ce que la couche séparatrice s’adapte d’une façon parfaite à la viande, il y aurait lieu au besoin de faire le vide dans les sacs. La congélation en sac une fois obtenue, les enveloppes se détachent aisément ; après quoi les morceaux congelés seront placés dans les chambres d’entreposage.”

Le dr. Heiss a effectué des essais sur de petits morceaux de viande, d’un poids pouvant atteindre un kg. en utilisant diverses enveloppes (caoutchouc, baudruche, toile huilée, soie imperméabilisée, cellophane reconnue perméable à la saumure).

Les réalisations mises au point par Mr. Hoveman portent sur les points suivants : fabrication d’enveloppes extrêmement fines à partir de latex brut, congélation rapide sous vide par ruissellement de saumure, stockage sous enveloppes protectrices à la même température, décongélation également sous enveloppe.

La préparation des sacs a soulevé de nombreux problèmes. Il fallait trouver une matière souple, très mince, étanche à l’air et aux liquides. Cette substance devait résister à toutes les aspérités : esquilles osseuses, irrégularités articulaires, arêtes de poissons, etc. Après de nombreux essais, le latex fut adopté comme base de fabrication en lui incorporant certains éléments conducteurs et désodorisants. Comme ces sacs doivent être considérés au titre d’emballage perdu, c’est-à-dire, utilisés jusqu’au moment de la décongélation du produit, ils devaient être d’un prix peu élevé. Les sacs en latex une fois préparés sont soumis à des tractions mécaniques progressives portant leur diamètre horizontal à un coefficient sept et leur diamètre vertical au coefficient dix. Chose curieuse, sous l’influence de la chaleur en particulier, ces augmentations peuvent être annulées. Ces modifications par tractions mécaniques sont donc réversibles.

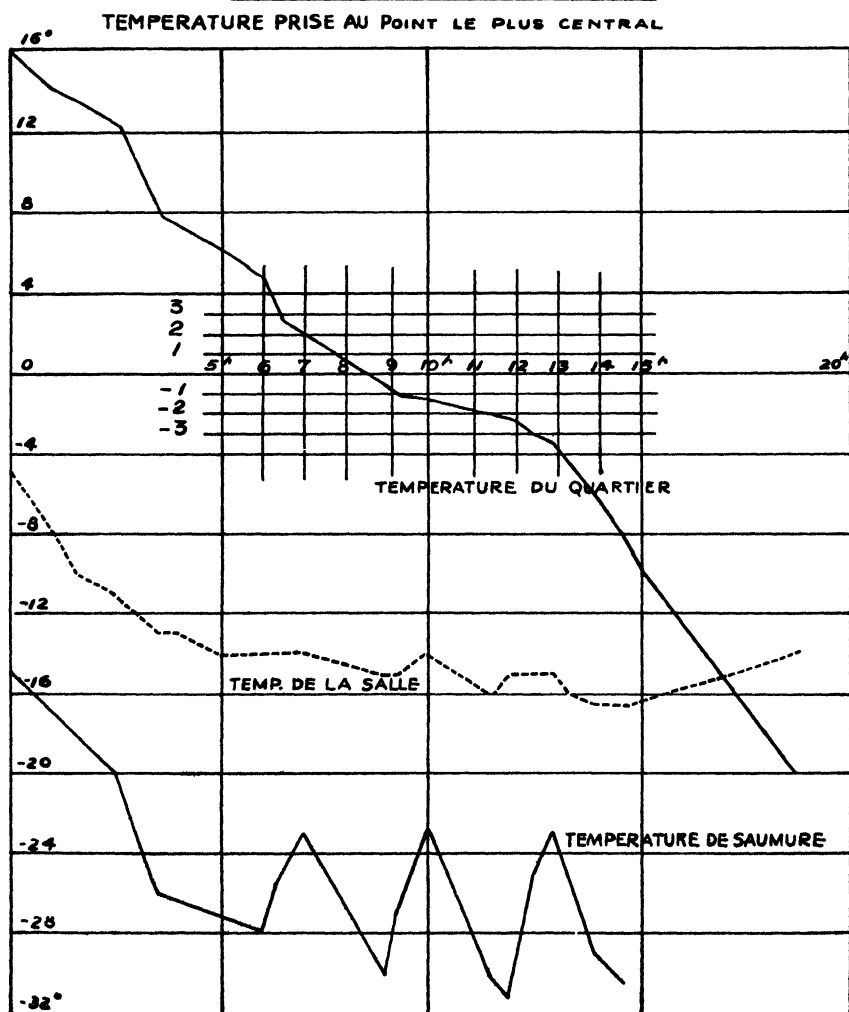
La congélation est obtenue, après adaptation parfaite, grâce à un bon vide, de l’enveloppe sur son contenu, supprimant toute paroi intermédiaire entre la viande et la saumure. Cette dernière d’une température de -26 à -30°C . (-14.8 à -22°F .) tombe en pluie sur

les quartiers de viande ou les carcasses de petits animaux suspendus à des crochets métalliques.

Nous avons assisté à plusieurs essais : congélation d'un coeur de boeuf encore chaud en 21 minutes, d'un foie en 22 minutes, d'un poulet de deux kilogrammes en 24 minutes, de douze truites introduites vivantes dans un sac en 21 minutes. La congélation d'une carcasse de mouton de 38 kg. a demandé trois heures et demie.

Voici le diagramme de congélation d'un quartier postérieur de boeuf de 55 kg. 500 préalablement rafraîchi aux environs de 16°C. (60.8°F.) (Essais pour l'armée) :

COURBE DE REFROIDISSEMENT D'UNE CUISSE DE BŒUF
POIDS : 55 kg. 500



La même technique permettrait également de préparer le *chilled beef* en utilisant alors de la saumure aux environs de -2°C. (28.4°F.).

La *décongélation sous enveloppe* nous a permis de faire quelques constatations des plus intéressantes.

D'une façon générale la présence d'enveloppe protège complètement son contenu contre la condensation de l'humidité extérieure de l'atmosphère et les souillures qu'elle entraîne : poussières, microorganismes de toutes sortes dont l'évolution ultérieure peut être si rapide. Les caractères des produits après décongélation suivant de près la congélation et libérés de leur enveloppe sont identiques à ceux des produits frais.

Les masses musculaires, les abats possèdent une belle coloration rouge, et les graisses la teinte habituelle, blanche ou légèrement jaune. L'ensemble a conservé intact ce que les Anglo-Saxons appellent le “bloom” des viandes. La consistance et l'odeur sont normales.

Les truites paraissent absolument fraîches, les écailles ont conservées leur brillant et leur reflet spécial, les ouies sont d'un beau rose, les globes oculaires blanc vitreux après la congélation, ont repris leur transparence.

Après six mois de stockage sous enveloppe, les viandes, la volaille n'ont subi aucune modification apparente. Des essais de dégustation, après cuisson, de filets de mouton ont été très satisfaisants, aucune saveur de rance.

La rancidité des graisses a été étudiée sur les quartiers postérieurs de boeuf conservés neuf mois et a donné les résultats suivants :

Acidité : pour cent en acide oleique :

Graisse des reins = 0 gr 49

Graisse de la face interne de la cuisse = 0 gr 559

Graisse profonde (ganglion poplité) = 0 gr 317

Les phénomènes d'oxydation sont donc presque négligeables.

Les résultats obtenus par Mr. Hoveman ont paru si intéressants à l'Intendance Militaire française qu'un premier marché de quelques tonnes de boeuf congelé lui a été confié tout récemment à titre d'essai.

L'exécution de cette première commande vient d'être réalisée aux Glacières d'Arras dans une petite installation provisoire comprenant les organismes suivants :

1. Couloir pour le rafraîchissement des $1/2$ carcasses de façon à obtenir une rigidité musculaire superficielle permettant la correcte séparation en quartiers, pour la mise sous enveloppe, et pour l'extraction des gaz à la pompe et la fermeture sous vide des sacs.

2. Chambre de congélation rapide par ruissellement de saumure à -26 à -30°C. (-14.8 à -22°F.) débitée par des rampes disposées au plafond et alimentées par un réservoir fortement isolé.

3. Chambre de stockage maintenue aux environs de -17°C . (1.4°F .) où sont arrimés les quartiers sous enveloppe.

L'ensemble de ces données permet d'entrevoir la possibilité d'appliquer industriellement le *quick freezing* à diverses denrées alimentaires et en particulier aux viandes, sous de grosses épaisseurs.

En dehors des très grands avantages particuliers signalés plus haut le point de vue économique paraît devoir être prépondérant. Si en effet la congélation des viandes qui actuellement exige de 72 à 80 heures peut être effectuée rapidement en 24 heures, il est facile de calculer les importantes économies apportées par le *quick freezing*: économie de temps, économie de cubage frigorifique entraînant une réduction très sensible des frais d'immobilisation et surtout d'entretien qui sont énormes dans les grandes usines d'exportation.

Les mêmes observations s'appliquent en outre au *quick freezing* qui paraît désormais possible.

L'Industrie Frigorifique peut trouver là des sources de progrès et de bénéfices intéressants.

CONTRIBUTION TO THE THEORY OF COLD INJURY TO FRUIT

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The preservation of fruit by means of cold storage is a much more complicated problem than the cold storage of meat, fish, and dairy products because each kind of fruit reacts differently and because the kind of soil, the annual climatic changes, and the environment in general exercise an influence on the product. Each country, therefore, must make its own experiences, and an application of the results obtained in other countries on their home production is permissible only to a very limited extent.

The favorable influence of low temperatures consists in retarding the ripening considerably; therefore, it is a rule to bring the fruit gathered in the correct state of maturity as quickly as possible to the refrigerator and to determine a lower limit of temperature only in so far as it is desirable to prevent fruits from freezing, since their tissues are thus destroyed and the cells killed.¹ It therefore seems practicable to keep a temperature from 0 to $-1^{\circ}\text{C}.$ (32 to $30.2^{\circ}\text{F}.$) in the fruit-storage rooms and, after gathering, to bring the fruit to this temperature as quickly as possible.

Numerous exceptions to this rule are known today, however, which become evident by the occurrence of cold injury to fruits attributable to disturbance of the normal metabolism even at temperatures above the freezing point. For example, citrus fruit must on no account be stored at temperatures below $5^{\circ}\text{C}.$ ($41^{\circ}\text{F}.$), and for subtropical fruits, such as bananas and mangoes, even higher temperatures are prescribed. A lowering of the storage temperatures of fruits is permissible only so long as thereby the numerous individual processes of the extremely complicated biochemical behavior will be retarded more or less equally. The normal development is then not disturbed but merely retarded. If the velocities of the simultaneous reactions interlocking with each other have very different temperature coefficients, certain substances can increase, whereas others decrease abnormally; thus a pathological state is brought about, causing undesirable changes in the taste in which the cells may be damaged or even killed. The best known example of a comparatively harmless injury from

¹ The author wishes to emphasize the fact that the present report is confined entirely to unfrozen fruits.

cold is the sweetening of potatoes at temperatures slightly above 0°C. (32°F.). At lower temperatures the velocity of sugar formation from starch is retarded less than the rate of decomposition of sugar owing to respiration, thereby resulting in an increased sugar concentration in the potato.

The temperature limit t_k , at which such pathological changes during storage set in, varies with the different species. It depends not only on the already mentioned factors but also on stage of maturity of the fruit at beginning of the cooling process. Internal breakdown progresses the longer fruit is subjected to the influence of deleterious low temperature.

Whereas hitherto it has been generally assumed that internal breakdown becomes more extensive the lower the temperature is dropped below the limit t_k , at which the first signs of injury become evident, the investigations made by Davies (1933-34, 1935) and his collaborators at the Low Temperature Research Station at Capetown have shown that for some South African varieties of fruit there exists a distinctly unfavorable temperature range for storage, roughly between 1 and 4°C. (33.8 and 39.2°F.). On storing below this temperature the rate of injury declines and may become almost negligible.

Recognition of this particular phenomenon is entirely new, although it is probable that other fruits may exhibit the same behavior, only with the difference that for most of them the unfavorable zone may occur only in the immediate proximity of freezing temperature or even below it, so that a possible decline of the injury at even lower temperatures could not be utilized from a practical standpoint.

The explanation of this phenomenon may be that although at lower temperatures the normal development may become more and more disturbed, the reaction velocities of the pathological metabolic changes decrease so much that for a certain period of storage the resulting deleterious effect is of lesser magnitude.

The behavior mentioned above was first ascertained in the case of several South African varieties of plums (Santa Rosa, Wickson, Gaviota, and Kelsey); it applies also to certain varieties of peaches.

The characteristic course of internal breakdown of Santa Rosa plums, as a function of temperature of cold storage for various stages of maturity A, B, and C respectively, at the beginning of cooling is shown (Fig. 1). The percentage of internal breakdown was ascertained when the fruit, during subsequent storage in a room at a temperature of 10°C. (50°F.), reached full table-ripeness or when the test had to be discontinued because the fruit, as in the state of ripeness A, would never reach full ripeness.

The absence of internal breakdown in itself is not the only criterion of the success of a cold-storage test. In addition it is necessary that fruit should normally ripen and develop its full flavor and coincident market value. Fruit in the stage of maturity A after three weeks in cold storage at a temperature of -1.5 and 1°C . (30.8 and 33.8°F .) did not attain ripeness at all; if the cold storage took place at 3 and 4.5°C . (37.4 and 40°F .), then after a further three weeks at 10°C . (50°F .), the fruit softened and turned red but its flavor value

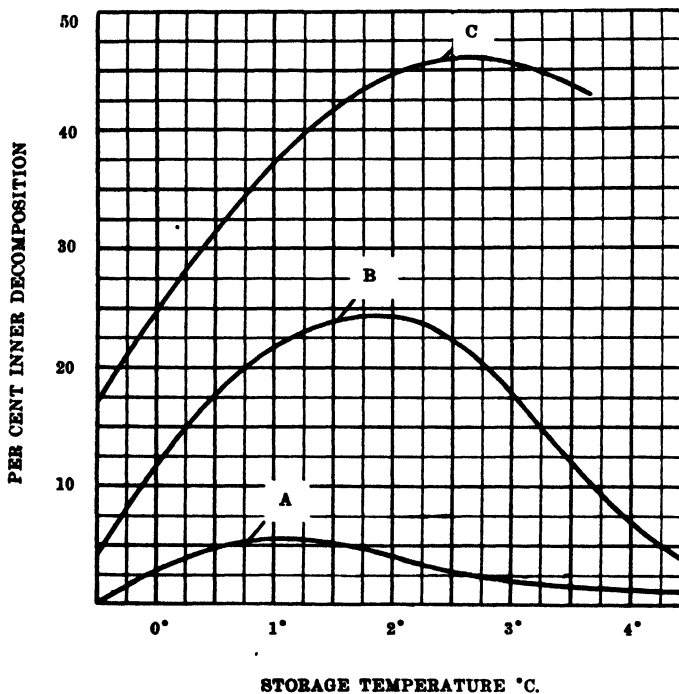


FIG. 1

remained poor. Fruits in the state of ripeness C (Fig. 1) suffered considerably from internal breakdown. On attaining table-ripeness the taste was often somewhat bitter and the flavor very unpleasant. The best results were obtained with fruit at the stages of maturity B and C after cold storage at -1.5°C . and in the stage of maturity B also after cold storage at 4.5°C . In the case of cold-storage temperatures above 6°C . (42.8°F .) cold injury no longer occurred, but then the fruit ripened so quickly in storage that its durability was insufficient for economic exportation.

It is now intended to calculate by means of a simplified model the biochemical changes taking place when fruit is exposed to low tempera-

tures and also to show that for a given period of storage in certain cases the injury may continue to increase with lower temperatures. It may likewise be shown that in other cases an increase of the injury is ascertainable only so far as a most unfavorable low temperature t_m is reached; further lowering the temperature has again an advantageous effect.

In the following kinetic observations it is assumed that the chemical processes in the living cell always occur at a great distance from equilibrium "for equilibrium is not life but death," according to Meyerhof (1926). The reaction velocity is then influenced mainly by the temperature, while the influence of the concentration changes can be neglected; for example, during the respiration of sugar in a fruit new sugar is always being formed from starch under the influence of diastase.

If the time is denoted by τ , the concentration of the reacting substance by c , and the initial concentration at the time $\tau = 0$ by c_0 , then there applies in the case of a monomolecular reaction the equation

$$c = c_0 e^{-k\tau}$$

in which k is the (so-called) velocity constant. By reaction velocity y is understood the quantity of substance converted in the unit of time,

that is the magnitude $-\frac{dc}{d\tau}$. Thus

$$y = -\frac{dc}{d\tau} = c_0 k e^{-k\tau} = kc \quad (1).$$

If during the reaction c does not perceptibly change, then the temperature has the same influence on the reaction velocity y as on the magnitude k .

Van't Hoff's (1903) rule, according to which the reaction velocity of chemical processes generally rises two or threefold if the temperature is raised by 10°C ., has been applied to the organic as well as inorganic nature. This rule accords with the equation formulated first by Berthelot (1882):

$$\log y = \log y_0 + a t \quad (2)$$

in which a is the temperature coefficient of the reaction velocity whereas y_0 represents the value of the reaction velocity at the tem-

perature $t = 0^\circ\text{C}.$ ² From equation (2) may be obtained for two temperatures $10^\circ\text{C}.$ apart, the following relation

$$\frac{y_{10}}{y_0} = \frac{y_{t+10}}{y_t} = \frac{k_{t+10}}{k_t} = 10^{10a} = Q_{10} \quad (2a).$$

For this velocity ratio the symbol Q_{10} has become customary in biology. Corresponding values of a and Q_{10} are given (Table 1). If then, according to van't Hoff, Q_{10} in general lies between 2 and 3, then the corresponding values of a lie between .03 and .05. In an extensive investigation of the respiration velocity of numerous varieties of fruits within a range of temperature of 0 and $35^\circ\text{C}.$ (32 and $95^\circ\text{F}.$) Gore (1911) found that, although y_0 varies considerably for the various kinds of fruit, the temperature coefficient a for all kinds of fruit investigated is approximately the same and its mean value, $a = .0376$ ($Q_{10} = 2.377$).

TABLE 1
Corresponding Values of a and Q_{10} According to Equation (2a)

a	.03	.04	.05	.06	.07	.08	.09	.10	.12
Q_{10}	2.00	2.51	3.16	3.98	5.01	6.31	7.94	10.00	15.85

The influence of temperature upon the formation of cold injury is investigated by the aid of the following simplified model of the biochemical behavior. Let us assume that in the ripening process following harvesting of the fruit, some harmful substances, such as alcohol, acetaldehyde, or ethylene, may be formed, acting as a cell poison within the fruit. Let us further assume that the formation velocity of such a substance is according to the following equation

$$\log y = \log y_0 + at \quad (2)$$

in which y ordinarily is measured in milligrams per kilogram of fruit per hour (Fig. 2). Let there be coupled with this process a second

² More exact than equation (2) is the relation given by van't Hoff and Arrhenius, cited by Eucken (1934)

$$\log y = B - \frac{A}{T} = B - \frac{A}{273} \cdot \frac{1}{(1 + t/273)}$$

For temperatures t in the neighborhood of $0^\circ\text{C}.$ the approximate equation is

$$\log y = B - \frac{A}{273} \cdot \left(1 - \frac{t}{273}\right) = B - \frac{A}{273} + \frac{A}{273^2} \cdot t$$

Putting $B - \frac{A}{273} = \log y_0$ and $\frac{A}{273^2} = a$ this equation is identical with equation (2). A and a are proportional to the heat of activation.

one in which the cell poison formed is decomposed by respiration. The velocity of this process may be determined by the equation

$$\log y' = \log y_o + a' t \quad (3).$$

It is now to be assumed that as temperature is decreased the rate of formation of cell poison decreases more slowly than its rate of decomposition, so that $a < a'$. Moreover as the measure of y or y' may be taken *ad lib.*, we put $y_o = 1$ and consequently $\log y_o = 0$.

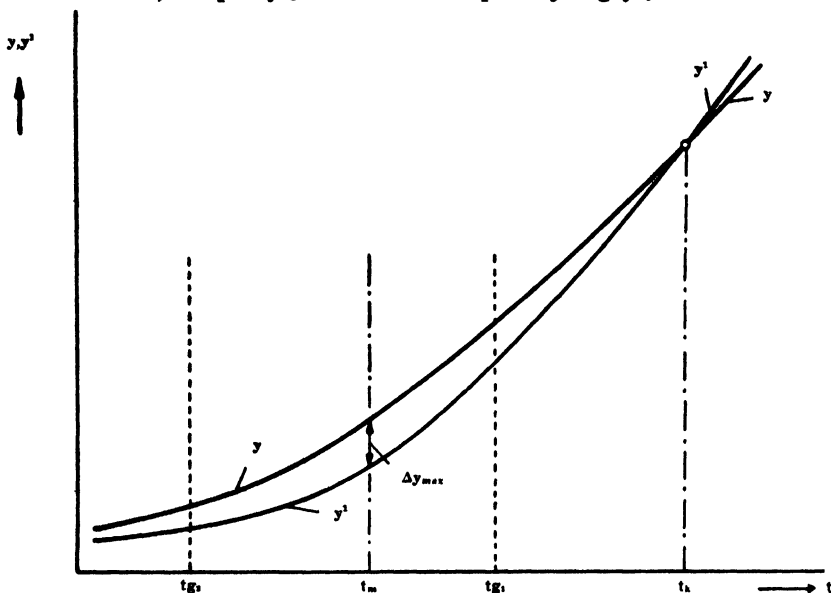


FIG. 2

At higher temperatures, at which $y' > y$, the cell poison formed is at once completely decomposed so that no injury can occur. The critical temperature t_k (Fig. 2), below which the cell poison begins to accumulate, is obviously given by the condition $y = y'$. According to equations (2) and (3) we find

$$\log y_o + at_k = a' t_k$$

and therefore

$$\log y_o = (a' - a) t_k.$$

Consequently

$$y = 10^{(a' - a)t_k + at}$$

and

$$y' = 10^{a' t}.$$

The quantity of cell poison that accumulates at a temperature $t < t_k$, which may be assumed as the measure of the magnitude of cold injury

(without, however, insisting on a proportionality between the two) thus is

$$\Delta y = y - y' = 10^{(a' - a)t_k + at} - 10^{a't} \quad (4).$$

It can now be easily demonstrated that this function has indeed a maximum for a temperature $t_m < t_k$, so consequently there exists an especially harmful temperature t_m . In precooling of fruit, this temperature will be obvious only in those instances when it lies above the freezing temperature t_g , since this limit should not be exceeded.

With the relation $\frac{d\Delta y}{dt} = 0$ for $t = t_m$, we obtain from equation (4)

$$a \cdot 10^{(a' - a)t_k + at_m} = a' \cdot 10^{a't_m}$$

and from that

$$t_m = t_k - \frac{\log(a'/a)}{a' - a} \quad (5).$$

If, for example, $t_k = 10^\circ\text{C}$. is assumed, we obtain for the various values of a and a' the values of t_m compiled (Table 2).

TABLE 2
Temperature t_m of Maximum Injury to Fruit for Various Values of Temperature Coefficients a and a' of the Interlinked Reactions at $t_k = 10^\circ\text{C}$.

a'	a	$\frac{\log(a'/a)}{a' - a}$	$t_m^\circ\text{C.}$
0.04	0.03	12.4	-2.4
0.06	0.03	10.0	0.0
	0.04	8.8	1.2
	0.05	7.9	2.1
0.08	0.04	7.5	2.5
	0.06	6.2	3.8
	0.07	5.8	4.2
0.10	0.06	5.55	4.45
	0.08	4.85	5.15
	0.09	4.5	5.5
0.12	0.08	4.4	5.6
	0.10	3.95	6.05
	0.11	3.8	6.2

From this can be seen that for values of a and a' between .03 and .05, which are in accordance with van't Hoff's rule, the temperature t_m of maximum injury lies close to the freezing point or even below it (freezing point t_g in Fig. 2). At falling temperature the cold-injury formation becomes stronger and stronger and may show a slight prac-

tically unimportant decrease only in the neighborhood of the freezing point. That is the behavior of most varieties of fruits investigated. To assume a temperature for t_k , higher than 10°C . does not appear permissible. (An exception is formed only by some kinds of tropical fruit.) Only for greater values of a' the temperature of maximum injury lies so high that a marked decrease in the accumulation of cell

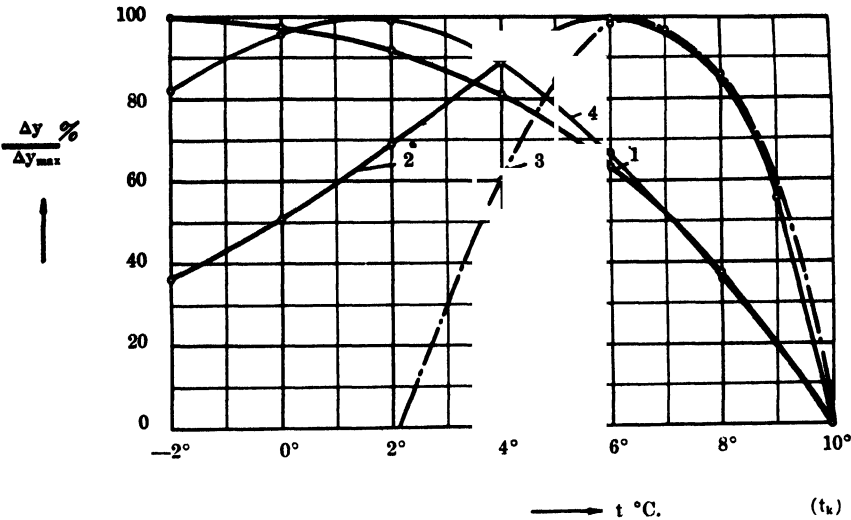


FIG. 3

poison can occur above freezing point (freezing temperature t_{k2} in Fig. 2). This is the case for South African plums (Fig. 1, Curves B and C).

The values of y, y' and Δy in two cases for temperatures from -2 up to 10°C . according to equations (2) to (4) are shown (Table 3).

TABLE 3
The Course of Fruit Injury as a Function of Temperature
(Q_{10} is constant)

Temperature in $^{\circ}\text{C}$.		-2	0	2	4	6	8	10 (t_k)
$a = 0.03$	y	1.096	1.259	1.445	1.660	1.905	2.188	2.512
	y'	0.832	1.000	1.202	1.445	1.738	2.089	2.512
	Δy	0.264	0.259	0.243	0.215	0.167	0.099	0
$a' = 0.04$	$\frac{\Delta y}{\Delta y_{\max}} \%$	100.0	97.9	91.9	81.3	63.1	37.4	0
$a = 0.11$	y	0.759	1.259	2.089	3.467	5.754	9.550	15.85
	y'	0.575	1.000	1.738	3.020	5.248	9.120	15.85
	Δy	0.184	0.259	0.351	0.447	0.506	0.430	0
$a' = 0.12$	$\frac{\Delta y}{\Delta y_{\max}} \%$	36.3	51.1	69.3	88.2	99.8	84.8	0

The values $\frac{\Delta y}{\Delta y_{\max}}$ for these two cases are shown as a function of temperature (Fig. 3, Curves 1 and 2).

It now remains to examine whether there occur practically (contrary to van't Hoff's rule) values of a and a' of .06 up to .12, which correspond to values of $Q_{10} > 4$ (Table 1). Indeed such values have been frequently observed in biochemical processes mainly and at low temperatures. Thus van't Hoff (1903) himself, and cited by Eucken (1934), mentions the case of an enzyme effect, in which $Q_{10} = 7.14$. Frequently Q_{10} depends on the temperature and greatly increases with falling temperature, in consequence of which the relations ex-

TABLE 4
Values of Q_{10} in Alcoholic Fermentation

Range of temperature °C.....	5 to 10°	10 to 15°	15 to 20°	20 to 25°
Q_{10}	7.02	4.45	3.24	2.46
Range of temperature °C.....	25 to 30°	30 to 35°	35 to 40°	
Q_{10}	2.04	1.82	1.44	

pressed by equations (2) and (3) lose their simplicity, and other temperature functions for y must be chosen, according to Plank (1937). Slator (1906), for example, has shown for alcoholic fermentation the values for Q_{10} (Table 4). These values can be easily represented by the following empirical formula

$$Q_{10} = \frac{60}{t+1} \quad (6).$$

If Q_{10} is calculated by means of this formula in the range of temperature of 0 to 5°C. (32 to 41°F.), important for cold storage (more exactly for 2.5°), $Q_{10} = 17$ is obtained. Bělehrádek (1935) likewise emphasizes that Q_{10} as a rule increases at falling temperature and surveys the explanations given therefor by various authors. According to Crozier (1924-25) in the formula of Arrhenius (compare footnote 2, page 179).

$$\log Q_{10} = \frac{10 A}{(T + 10) T} \quad (6a).$$

A reaches values up to 7,600; at $T = 273^\circ$ ($t = 0^\circ\text{C.}$) the corresponding value of Q_{10} is nearly 10. Halban (1909) is of the opinion that high values of Q_{10} (up to 6) are characteristic for monomolecular reactions. Also in animal metabolism high Q_{10} values have been found, for example, for the oxygen consumption between 10° and 15°; ($Q_{10} = 5.7$ was found); for the gas-change of marmots at 10°C.,

Q_{10} is even as high as 13 to 16, according to Kanitz (1915). Occasionally much higher values of Q_{10} are found, such as for the denaturation process of a protein (leucosin), the value of $Q_{10} = 48$, according to Lüers and Landauer (1922). This shows that in biochemical processes there are not seldom such high values for Q_{10} and for a . According to our theory a clearly marked maximum in the formation of cold injury at low temperature may be expected in such cases.

In the event of Q_{10} not being constant, the place of equation (2) is taken by another function $y = f(t)$ and the character of the curves (Fig. 3) changes. Q_{10} , y , and t are connected with each other by the differential equation, according to Plank (1937)

$$\ln Q_{10} = 10 \frac{d \ln y}{dt} \quad (7).$$

If for the formation of cell poison according to equation (6) we put

$$Q_{10} = \frac{B}{t + c}$$

we find from equation (7) by integration

$$y = y_0 \left(\frac{c}{t + c} \right)^{c/10} \left(\frac{e B}{t + c} \right)^{t/10} \quad (8)$$

in which $e = 2.718$ is the basis of the natural logarithms. At falling temperature y then declines much more rapidly than would be the case if Q_{10} were independent of temperature. If for the cell-poison formation we take in equation (8) $B = 40$ and $c = 10^\circ\text{C}$., we find

$$y = y_0 \left(\frac{10}{t + 10} \right) \left(\frac{108.72}{t + 10} \right)^{t/10} \quad (8a).$$

If we are to proceed from the same value of y (and y') for $t_k = 10^\circ\text{C}$. with which we have hitherto compiled (Table 3) for $a = .03$ and $a' = .04$, that is $y_{tk} = 2.512$, from equation (8a) is now obtained

$$y_0 = .925$$

in the place of $y_0 = 1.259$ (Table 3). Thus y decreases now much more rapidly. If it is further assumed that the law of the respiration of cell poison remains unchanged, so that equation (3) and the values of y' (Table 3) remain valid, then we obtain for $\Delta y = y - y'$ the values compiled (Table 5).

The values of $\frac{\Delta y}{\Delta y_{\max}}$ are entered (Fig. 3) as Curve 3. It is now

seen that at temperatures below 2°C. (35.6°F.) no cell poison can accumulate and therefore in this sphere no cold injury can occur.

Finally it can be considered that the cell poison which accumulates in the course of the storage increases the rate of respiration. We assume according to equation (1) a proportionality between the quantity y' respired in the unit of time and the concentration. A certain amount of cell poison y_n (in mg. per kg. of fruit) may nor-

TABLE 5
The Course of Fruit Injury as a Function of Temperature
(Q_{10} is dependent on temperature)

Temperature in °C.	0	2	4	6	8	10
y acc. to eq. (8a)	0.925	1.198	1.498	1.825	2.165	2.512
y' acc. to eq. (3)	1.000	1.202	1.445	1.738	2.089	2.512
Δy	-0.075	-0.004	0.053	0.087	0.076	0
$\frac{\Delta y}{\Delta y_{\max}}$ %	(-4.53)	59.8	98.4	85.9	0

mally be present and the velocity of respiration y' (in mg. per kg. fruit per hour), up to now calculated, may correspond to it. If now, below the temperature t_k the cell poison accumulates to higher values, the velocity of respiration may increase to a value y'' .³ If the formation velocity of the cell poison is, as hitherto, y , then a quantity $(y - y'') \tau$ in mg. per kg. of fruit accumulates in the storage time τ . We have therefore the relation

$$\frac{y''}{y'} = \frac{y_n + (y - y'') \tau}{y_n}$$

or

$$y'' = y' \left(1 + \frac{(y - y'') \tau}{y_n} \right) \quad (9).$$

³ These assumptions correspond to the process of respiration of sugar in potatoes. Potatoes normally have a sugar content of about one per cent. If they are stored at temperatures below 3°C., the sugar content increases as the storage temperature is lowered. At the same time the respiration rises; it is greatest in potatoes rich in sugar, though the low temperature in itself hinders the respiration (R. C. Wright).

If now we put the quantity of cell poison, collecting in the unit of time, $y - y'' = \Delta y''$, we find

$$\Delta y'' = y - y'' = y - y' - \frac{y' \cdot \Delta y'' \tau}{y_n}$$

whereof we get with the hitherto existing value $\Delta y = y - y'$ the relation

$$\Delta y'' = \frac{\Delta y}{1 + \frac{y' \tau}{y_n}} \quad (10).$$

As now we have to assume $\Delta y''$ instead of Δy as the measure for the formation of cold injury, it is seen that with the foregoing generalized suppositions a lesser amount of cold injury is always to be expected. The amounts do not, however, diminish in the same degree at differ-

TABLE 6
Course of Injury of Fruit as Function of Temperature
(The cell poison accumulated increases the rate of respiration)

Temperature in °C.	-2	0	2	4	6	8	10
$\Delta y''$	0.130	0.150	0.156	0.140	0.106	0.057	0
$\frac{\Delta y''}{\Delta y''_{\max}} \%$	82.5	95.3	99.0	88.9	67.3	36.4	0

ent storage temperatures, as y' is still dependent on temperature, so that the position of the maximum (Fig. 3) can also be shifted.

If we now take as example the second case (Table 3) ($a = .11$ and $a' = .12$) and insert the therein contained values for y' and Δy in mg. per kg. of fruit per hour into equation (10), we get, with $y_n = 1,000$ mg. per kg. of fruit for a storage time of one month the values entered (Table 6). The real values for y' , Δy , y'' and y_n may of course be only a fraction of those mentioned here and based upon the arbitrary assumption that $y_0' = 1$ at $t = 0^\circ\text{C}$. This process is entered (Fig. 3) as Curve 4. As is seen, the position of the maximum in relation to Curve 2 has shifted from 6 to 2°C . and the maximum has become much more even.

These considerations may serve as a first step to a physico-chemical theory of cold injury. Our knowledge of the chemical processes taking place during normal or pathological metabolism is still so limited that a complete theory of cold injury in my opinion cannot yet be elaborated.

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MICROBIOLOGY IN RELATION TO FOOD PRESERVATION ✓

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With the great advances in food technology, knowledge of the effect of various processes on microorganisms causing fermentation, deterioration, or spoilage of food materials has assumed great significance. Of like importance is knowledge of the effect of these processes on organisms capable of bringing about so-called food infections in man, since the possible widespread occurrence of *Clostridium botulinum*, *Salmonella* species, and other organisms concerned is now more fully recognized than was the case even a decade ago. As a result of studies in food bacteriology we are now familiar with the fact that the character of decompositions may be influenced by the initial bacterial flora of food itself or by contaminations it receives during handling and preparation for treatment. The food technologist must, therefore, be aware of inherent or potential dangers of deterioration owing to natural association of bacteria and raw materials, as well as of the germ-destroying or inhibiting nature of the process of treatment.

In these matters the experience of the past has been of great value. Established practices in the treatment of foods gave fairly adequate assurance of successful conduct of many processes long before the scientific theory had been tested and definitely established. Food preservation by drying, salting, smoking, and use of sugar, vinegar, or spices has been known and practiced with reasonable success since very ancient times. The exact knowledge of the inhibitory nature of some of these processes owing to changes in physical state, or of the germicidal character of others owing to the presence of antiseptic chemical substances, was entirely unknown to those who employed these primitive methods because they had no knowledge of microbes. Discovery, study, and organization of increasing knowledge regarding molds, yeasts, and bacteria led to recognition of the part played by microbes in food deterioration and spoilage, and later to distinction between those which could induce various types of destructive action.

That occasional organisms were present which could endure or even grow in conditions ordinarily restrictive, as in high salt content

or presence of considerable concentrations of sugar or vinegar, was discovered only after careful investigation of processes had begun. In all these fields research has yielded important results.

FOOD PRESERVATION METHODS

It is convenient to divide methods of food preservation into two classes: those which are essentially inhibitory or germ-restraining and those which produce germ destruction or sterilization. In all the former, one operates on the principle that while the reduction in numbers or kinds of bacteria may be brought about to a greater or less degree, a residual population of living yeasts, mold spores, or bacteria always remains in inactive condition. Obviously these vary greatly according to the character of the food and the process employed.

Use of controlled temperatures provides processes in which both of these principles are exemplified. Furthermore, of all processes of food preservation those involving temperature relations have proved of greatest importance up to the present time. The procedure of Nicolas Appert was the forerunner of the methods of heat treatment which we have come in recent years to consider under the name of sterilizing processes in which the aim is to bring about complete destruction of microbial life, although it may not always be attained by action of relatively high temperatures, as in canning. These processes have in the past five decades received great attention and publicity, and need not be further discussed at this point except to say that new modifications involving the use of still higher temperatures and shorter periods of heating are now receiving thoughtful consideration.

Preservation of food by low temperatures, although not new, is one of the most striking and important aspects of present-day food technology. Although in commercial use for more than 70 years, refrigeration of foods has constantly assumed new importance because of great improvements which have been made in equipment and in technique of refrigeration processes whereby extremely low temperatures may be secured. As a result there has been developed in recent years not only the process of "quick freezing" but great advancement in methods of transportation of foods under refrigeration, in general cold storage, and in domestic or household refrigeration. While these great improvements have been largely in the mechanical field and are a result of research on new refrigerants, great improvement has also occurred in application of either natural or artificial ice as a result of new design and improved insulation of the food-storage cabinets or chambers in which ice is the refrigerant. Regardless of the nature

of the refrigerant used, the food technologist is always confronted with one important question, viz., what is the effect of the temperature attained on viability of microörganisms in or upon food treated or maintained at low temperatures?

This question may be still further broken down into two subsidiary inquiries: (1) What is the effect of low temperatures on pathogenic bacteria that might gain access to food? (2) What is the effect on organisms of spoilage and on ordinary and relatively inactive saprophytes which are present in or upon food material refrigerated?

It may be granted without argument that from the standpoint of preventing undesirable tissue changes, use of low temperatures or rapid freezing provides an ideal method for preservation of meats and fish and for many fruits and vegetables, provided high-grade, wholesome, raw materials and varieties suitable for these treatments are used. Much evidence has accumulated both in America and in Europe that not all types of fruits are equally adapted to low-temperature treatment. This may not, however, be a matter of microbial association or invasion but one of inherent structural and enzymatic properties of the food substance itself.

We are here concerned with the effect of low temperatures on microbes themselves, bearing in mind the possibility that these may be of many kinds and may even include pathogenic types as a result of insanitary handling, water or soil infections, or other causes. While we may accept the usual generalization that low temperatures inhibit bacterial or microbial growth, it is important to point out that there are two factors which the food technologist should not overlook. Not all bacteria, yeasts, or molds, even of the same germs or species, will behave alike in this respect. In other words there are some strains which are more resistant or more adaptable to the low-temperature environment than others. In general, at the freezing point many may be killed; at lower temperatures, more, but some will almost surely survive. The death curve is similar to the curve for heat treatment or for action by a disinfectant but in general runs at a different level. There is the fairly rapid falling off in numbers in the early part of the treatment, followed by more gradual reduction and eventual elimination of all except a few persistent survivors.

For certain strains or species, however, it may happen that if the material is not actually frozen solid the organisms become adapted to low temperature after a time and slow growth may take place, increasing materially with the lapse of time, until the numbers of organisms may reach large figures. While this is characteristically true in cold storage above the freezing point, it is not likely to occur frequently at lower temperature ranges.

EFFECT OF FREEZING ON PATHOGENIC BACTERIA

The effect of freezing on pathogenic bacteria has been studied from time to time for 40 years. Investigations of the effect of freezing on typhoid bacilli by numerous investigators have shown that about 99 per cent are destroyed in the original freezing when the germs are suspended in water, although it has been shown by Tanner that freshly isolated strains of the typhoid bacillus apparently have much greater resistance to freezing than have laboratory strains which have been grown on artificial media for several generations. The disparity between the numbers of organisms used for inoculation experiments and the number surviving indicates that the dangers from this source, although existent, are relatively slight. These facts might be of significance in case an unsuspected typhoid carrier or a person suffering from the disease were engaged in actually handling food materials in preparation for freezing, or if such materials were washed in polluted water containing pathogenic organisms.

It has been shown, furthermore, by inoculation and storage experiments that the extremely few survivors may remain alive at 0°C. (32°F.) for three to five weeks. Whether they still remain infective or virulent is, of course, unknown. In the presence of food materials this period of survival may be greatly increased, for example in ice cream, but the percentage of surviving organisms is still extremely small. Temperatures below 0°C. appear to be not distinctly more lethal for all organisms than the freezing point itself. All the available evidence indicates that there is little if any danger of typhoid from food substances which are actually frozen and stored at temperatures below 0°C. unless the infection is massive in character. We are unable to discover any outbreak or case of the disease traceable to cold storage or frozen foods, with the possible exception of ice cream.

EFFECT OF FREEZING ON BACTERIA IN FOODS

Investigations of the survival of several *Salmonella* species and of *Clostridium botulinum* using large dosages for the original inoculations in foods have shown that very small numbers of these organisms can survive for periods of several weeks at temperatures as low as -20°C. (-4.0°F.) to -40°C. (-40°F.). In connection with studies on *Clostridium botulinum*, the most important fact to be determined is whether toxin formation can take place; and second, if toxin can develop when frozen or refrigerated foods in which spores of this organism exist are kept at higher temperatures before use. Researches were made in the laboratory by Prescott and Geer (1936), in which packaged spinach, heavily inoculated with detoxified spores of Type A *Clostridium botulinum*, was frozen at -15 to -30°C. (5 to -22°F.)

and later kept at refrigerator temperatures below 10°C. (50°F.), at 10°C., and also at 20°C. (68°F.) and above. A determination of development of toxin was made by animal inoculation into healthy white mice. The results may be summarized as follows:

At temperatures considerably below 10°C. no development of toxin occurred during the period of observation (more than one month) in either inoculated or uninoculated samples.

At 10°C. no toxin development occurred within a period of 31 days. At this temperature, however, the raw spinach became so spoiled by other organisms that it was obviously inedible. Similar results as to spoilage were shown by the uninoculated control packages.

At 20°C. decomposition occurred in two to four days in all packages, and toxin production also was demonstrated in the inoculated packages.

At higher temperatures both spoilage and toxin production occurred rapidly in the inoculated specimens.

With peas and other vegetables and fruits similar results were obtained, although it was found that the production of certain acids by other bacteria normally occurring on the material used tends to delay, or in some instances to inhibit, toxin production at 20°C. The same fact was noted with fruits or vegetables having pronounced natural acidity.

From this work it seems apparent that when *Clostridium botulinum* in foods is subjected to "quick freezing" and to storage at temperatures below 10°C., toxin production is inhibited for at least 30 days, and that the lower the refrigeration temperature the greater the protection against danger from *Clostridium botulinum*. As a corollary, the effective refrigeration at or near the freezing point is to be recommended in case of all foods which have not been heated thoroughly in which this organism might be present.

Studies with several strains of *Salmonella* indicated that refrigeration at 5°C. (41°F.) or less must be employed to ensure that these food-poisoning organisms will not grow in foods. Our results indicated a sharp falling off in numbers at temperatures below this point.

The effect of low temperature on the organisms of ordinary spoilage and on saprophytes in general is also strongly restrictive but varies in different foods and with different organisms. The important thing which we would emphasize is the selectivity shown when temperatures of 0 to 5°C. (32 to 41°F.) are employed. In our experience the protein attacking types can develop while the carbohydrate fermenters are suppressed.

In work carried out a few years ago with a variety of foods kept under refrigeration, Bates and Prescott (1929) found the same type

of results, which were especially observable in slime-forming organisms on beef. It was evident also that the relative humidity and rate of air movement is of importance. With food materials handled rapidly and in a sanitary manner and treated at once by the methods of "quick freezing" the situation is somewhat different. The much lower temperature applied here acts as a real agency of destruction to many kinds of microorganisms so that "quick frozen" foods in general show much smaller numbers of viable bacteria, yeasts, and vegetative molds. Undoubtedly this is due in a measure to actual freezing of the germs themselves and to the resultant disturbance of colloidal masses within the cells. This disturbance is probably less in the case of spores of bacteria and molds, if our general assumption that these are partially dehydrated structures is justified. It is an observable fact, however, that the organisms which survive extended periods at these low temperatures are not all spore-forming types.

Death rates of bacteria at temperatures below freezing are markedly influenced by the conditions which obtain. Consequently, generalizations as to what might hold for foods should not be made from data secured with pure cultures in watery suspensions. Wallace and Tanner (1935) found that death rates of microorganisms increased with increasing acidity (pH 4.0-9.6). Amounts of salt from one to six per cent and of sugar from 10 to 50 per cent did not seem to have much effect on pure cultures.

Botulism is no more of a threat from frozen foods than it is from foods preserved by other methods. Since it is a menace to foods preserved in many different ways, however, its possible relation to frozen foods should be analyzed. Consideration of these possibilities may also prevent trouble from this angle. As far as the authors know frozen foods have not caused botulism. The nearest approach, perhaps, is botulism attributed to an imported canned fish product which had not been sterilized in the can and which was intended to be handled under refrigeration. The product had not been properly stored and was decomposed, as indicated by the condition of the container. This cannot then be offered as a botulism outbreak caused by a frozen food. It may, however, be offered as an example of what might happen, if frozen foods are not properly handled.

In addition to the studies mentioned above, the influence of freezing on *Clostridium botulinum* has been studied by various bacteriologists: Wallace and Park (1933), James (1933), Straka and James (1932), and Tanner and Wallace (1931). The last-mentioned investigators showed that the spores of *Clostridium botulinum* survived freezing at $-16^{\circ}\text{C}.$ ($3.2^{\circ}\text{F}.$) for 14 months. When the vegetables were

thawed at room temperature, they became toxic in from three to six days.

Interesting results were secured when detoxified spores of *Clostridium botulinum* were added to fruits just before freezing. Despite a pH which would ordinarily protect from botulism, thawed frozen fruits became toxic in a few cases. Their hydrogen ion concentration would ordinarily be believed to protect from botulism. A pH is ordinarily accepted as the critical one, on the acid side of which there is usually protection. This was established, it should be pointed out, with heat-processed foods and for an environment in which *Clostridium botulinum* was the only organism. Presence of other organisms, as would be the case with frozen foods, might make it possible for *Clostridium botulinum* to form its toxin. There has been too much tendency to compare frozen or frosted foods to foods preserved by other methods.

Little hazard of botulism from frozen foods exists if those who use this method of preservation realize their responsibility to use only fresh, clean raw foods and to see that they are kept frozen until used by the purchaser. Some risk is introduced if they are allowed to thaw before use. Fortunately, most foods which may be significant in this connection are thoroughly cooked in preparation for the table and thus the toxin is destroyed. Repeated thawing and freezing are always objectionable as such treatment causes loss of quality.

BEHAVIOR OF MICROÖRGANISMS BELOW FREEZING

The behavior of bacteria, yeasts, and molds below freezing has not been intensively studied. The literature contains a few statements to the effect that microörganisms below zero in the frozen state are dormant because they are really in a dry environment. Moisture is crystallized as ice and is, therefore, not available for metabolism. So far as we know microörganisms do not have a body temperature which would make any water available. It has been generally assumed, therefore, that microörganisms cannot grow below zero. Whether this assumption is valid depends on many different factors; presence of dissolved salts may so lower the freezing point that some of the water is not crystalline and might be available to microörganisms.

While some who have followed the microbial content of ice cream have reported decreases in numbers of microörganisms, several have reported increases in the numbers of bacteria. Weinzirl and Gerde-man (1929) reported that storage of ice cream at $-10^{\circ}\text{C}.$ ($14^{\circ}\text{F}.$) does not prevent increase in the bacterial count. Ellenberger (1919) also reported evidence that bacteria might increase in stored ice cream.

These differences in result may sometimes be ascribed to methods employed.

Berry (1933a) reported growth of *Cladosporium* sp. at -2°C . (28.4°F .) and of *Oidium* sp. and *Torula* sp. at -4°C . (24.8°F .) on small fruit in non-air-tight containers. He also presents evidence that *Lactobacilli* and *Aerobacter* will persist in peas stored at -10°C . for at least two years, *Aerobacter* in string beans for 19 months and in spinach for 10 months, and that bacteria of the genus *Pseudomonas* will increase when stored at -4°C .

The growth of microorganisms at -4°C . (24.8°F .) and at -6.7°C . (20°F .) has been reported by Berry (1934). Those growing at -4°C . were *Pseudomonas fluorescens*, *Lactobacillus* sp., *Torula* sp., *Monilia* sp., and *Penicillium* sp. *Pseudomonas fluorescens*, *Lactobacillus* sp., and *Penicillium* sp. were isolated from scalded or blanched peas packed with three per cent NaCl solution and stored at -4°C . *Pseudomonas fluorescens* was inoculated into sterile pea broth and nutrient gelatin, and growth was observed after four weeks. Slight growth of *Lactobacillus* sp. in gelatin could be seen after six weeks' incubation. Mold growth of *Penicillium* sp. was observed after three months on the outside of several of the paper containers in which the peas were packed and stored. *Torula* sp. was isolated from strawberries packed in 50 per cent sucrose solution after four weeks' storage at -4°C . Transfers on nutrient agar yielded pink colonies after two weeks' incubation. *Monilia* sp., isolated from the same strawberries after six weeks, showed growth on wort agar transfers after eight weeks. The microorganisms growing at -6.7°C . were *Cladosporium* sp. and *Sporotrichium* sp. *Cladosporium* sp. was isolated from okra, packed with three per cent NaCl, and from kale, both having been stored for 15 months at -6.7°C . *Sporotrichium* sp. was isolated from scalded peas packed in three per cent NaCl and stored for 10 months. Transfers of small mycelial tufts and spores of both were made into sterile peas in three per cent NaCl solution and incubated at -6.7°C . Growth appeared in three months on the peas and after four months on the surface of the frozen brine.

From frozen strawberries, raspberries, and cherries held in storage at -9.4°C . (15°F .) for three years, Smart (1934b) isolated 26 species of bacteria, yeasts, and molds.

It is impossible in the scope of this general paper to refer specifically to all the excellent investigations on the microbiology of refrigerated or frozen foods.

The microbiologist has added greatly to knowledge of fundamental character in all the fields of food preservation and has done great service both from commercial and public-health standpoints. The food

industry should recognize that he is essential to proper food manufacture and that his work must be continuous and expanding, for certainly many new avenues of discovery and usefulness will lead from the work already accomplished in all the fields of food preservation.

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DETERMINATION OF OPTIMUM CONDITIONS FOR DOMESTIC REFRIGERATION OF FOODS

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Several papers presented at this Food Technology Conference have indicated the extensive utilization of modern refrigeration for preservation of a wide variety of foods, either during transportation, manufacture, storage, or distribution. In each instance the primary objective was to safeguard healthfulness, quality, and economic value of the particular edible commodity until it reaches the American home.

It is equally essential that proper domestic refrigeration facilities for storage of perishable foods be available in the home or in those establishments where such foods are to be consumed if the final link in the chain of food protection is to be as strong as the rest of the chain.

The question as to what constitutes proper refrigeration facilities has been subject to much discussion and investigation. Doubtless all concerned will agree that "proper facilities for domestic refrigeration" should be those which at least approach what we may call optimum conditions for temporary preservation of those foods for which such facilities would normally be used. Certainly it is the goal toward which investigations in this field should be directed.

Over a period of years a series of investigations has been conducted on various phases of domestic food preservation by Prof. S. C. Prescott and his collaborators. Some of this work has been concerned with the study of optimum conditions for temporary or domestic food refrigeration and the present discussion will be limited to certain aspects of a part of these investigations, which have been conducted by the Massachusetts Institute of Technology, Division of Industrial Coöperation, as a joint research with certain refrigeration industries.

If one considers the obvious changes that may occur in the home storage of perishable foods that render such substances less desirable or unfit for use, it is likely that some of the following criteria would be involved: changes in color, flavor, taste, or odor; surface drying; slime formation; mold growth; changes in texture; surface hardening; changes in turgidity or limpness. Weight changes are also likely to occur, although such losses are doubtless of less significance to the housewife than to the food manufacturer or distributor. In addition to these factors there is the definite possibility of changes which are

not detectable by the senses unless such deterioration has progressed to an advanced stage, previous to which time only bacteriological or chemical tests may be used as an index of the extent to which changes have taken place.

The object of these particular experiments was to determine whether any limits might be observed with respect to air temperature, relative humidity, and rate of air motion which could be considered a common optimum zone for the preservation of all the test foods under investigation. It was realized that any such combination of conditions would in effect be a compromise, as the optimum storage condition for any one food sample of either plant or animal origin depends on its nature, composition, natural microbial infection, cellular structure, surface area, volume, and similar factors.

An effort was made to select a number of common foodstuffs which are frequently found in domestic refrigerators and subject them to carefully controlled and known refrigeration conditions in order that comparisons might be made concerning the effects of various storage conditions on the changes which occurred in the foods. The foods selected for these experiments included uncooked pork chops, lamb chops, and hamburger steak; raw beef liver slices; uncooked haddock fillets; canned peas; boiled, peeled potatoes; whole heads of lettuce; and bunches of celery.

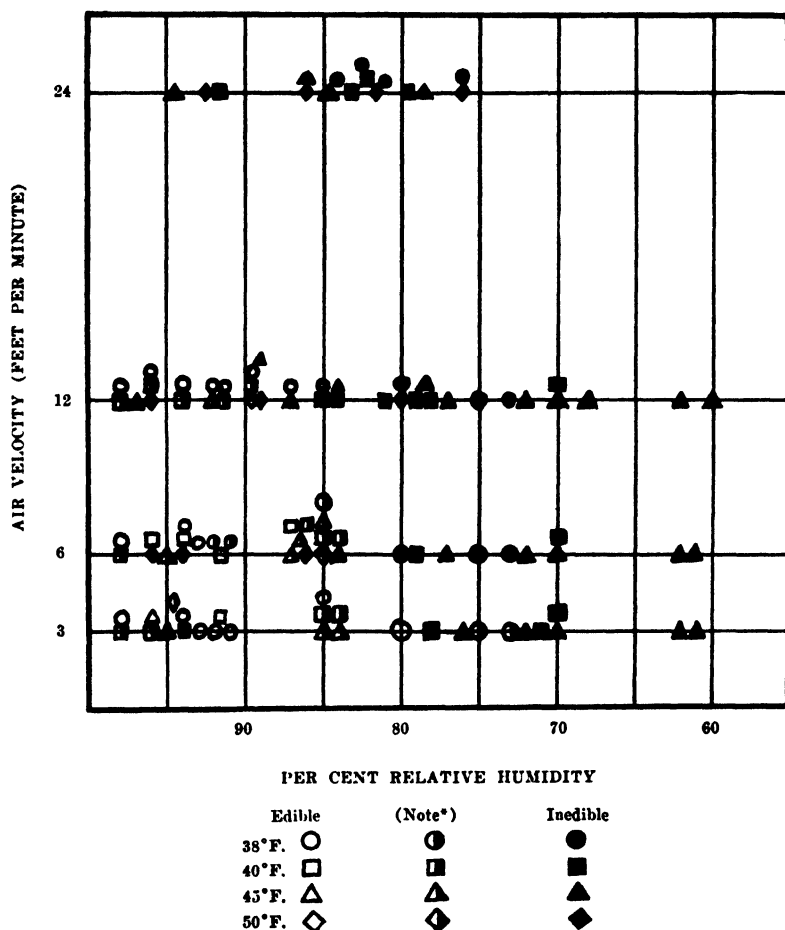
These foods were subjected to storage in test chambers¹ in a refrigerated, insulated test room capable of close temperature regulation. In this room were six test chambers, constructed in units of three each. The chambers had a capacity of approximately four cubic feet and contained two shelves with a total shelf area of 4.5 square feet.

The unique feature of these chambers was the fact that each unit had an independent air-circulation system capable of furnishing air at a definite temperature of approximately the desired relative humidity, with the simultaneous possibility of controlling the rate of flow of air in each chamber so that the foods in each of the three chambers might be subjected to a different measured rate of air flow. The adjustment of humidities within limited ranges was obtained by passing the air through scrubbing towers in which it was passed counter-current to a saturated solution of the particular salt necessary to produce the humidity desired. Precautions were taken to prevent fine droplets of the salt solution from entering the chambers

¹ This test equipment was designed and fabricated under the supervision of Dr. Philip K. Bates of the Department of Biology and Public Health with the cooperation of a committee of staff members of the Departments of Mechanical Engineering and Chemical Engineering of the Massachusetts Institute of Technology.

and the humidified air was blown into them at the bottom and withdrawn at the top of each, thereby enabling a recirculation of air through the humidification system.

In each of these cabinets a number of thermocouples were installed on the different shelves and in different sections, and humidity read-



Note.* In such cases, duplicate runs produced at one time edible hamburger, and at another time, hamburger unfit for use.

FIG. 1. Appearance of hamburger steak at the end of four days' storage.

ings were made by means of hair hygrometers which were calibrated at the beginning of each test by means of an electric aspirating psychrometer.

The foods used in these experiments were purchased in local retail stores immediately before the start of each test and efforts were made

to have samples as nearly comparable as possible. A four-day storage period was used in each instance and the foods were placed in dishes to simulate the conditions of home storage. The time period used was considered to be much more severe than one would expect in ordinary households, however.

Some 45 runs have been completed with this equipment to date, using temperatures ranging between 2.2 and 10°C. (36 and 50°F.), air velocities of 3, 6, 12, and 24 feet per minute, and relative humidities which ranged from 61 to 98 per cent in various combinations.

Certain conclusions may be drawn as a result of these storage experiments when the foods were evaluated at the end of the test runs by the criteria of appearance, acceptability as food, and edibility.

It was apparent that for the particular food materials which comprise the group under investigation, temperatures of 4.4°C. (40°F.) or lower provided the more satisfactory storage conditions. A graphical representation of the results obtained with hamburg steak under different storage conditions may be considered as typical of the findings relating to a number of the foods investigated (Fig. 1).

Air velocities in the 12 feet per minute range appear to provide satisfactory storage conditions for this group of foods if the above temperature factors are observed and the relative humidities are maintained above 92 per cent.

Satisfactory storage conditions for this combination of foods were also evident when an air velocity of six feet per minute was combined with relative humidities of 85 per cent and higher, at temperatures of 4.4°C. (40°F.) or below.

Lower air velocities, namely, at the rate of three feet per minute, appear to be better adapted to the storage of the plant materials utilized in these experiments than for animal tissues, which frequently showed evidence of bacterial slime formation.

Air velocities at the rate of 24 feet per minute were not satisfactory for any of the test materials used in the range of relative humidities used, namely, 75 to 95 per cent.

The air-flow conditions between 12 feet per minute and 24 feet per minute have not as yet been investigated.

The important effect of temperature on bacterial growth rate (Fig. 2) indicates the same general trends that have been observed in respect to a number of other food products. The retarding effect of the lowest temperature used in this particular experiment, 3.3°C. (38°F.), in comparison with those storage temperatures of a higher order are quite evident.

Some experiments which we have conducted to determine the effect of temperature on the vitamin C content of vegetables indicate

that even for short-time storage of such foods as spinach, temperatures below 4.4°C. (40°F.) tend to conserve this vitamin, which deteriorates rapidly at ordinary room temperatures.

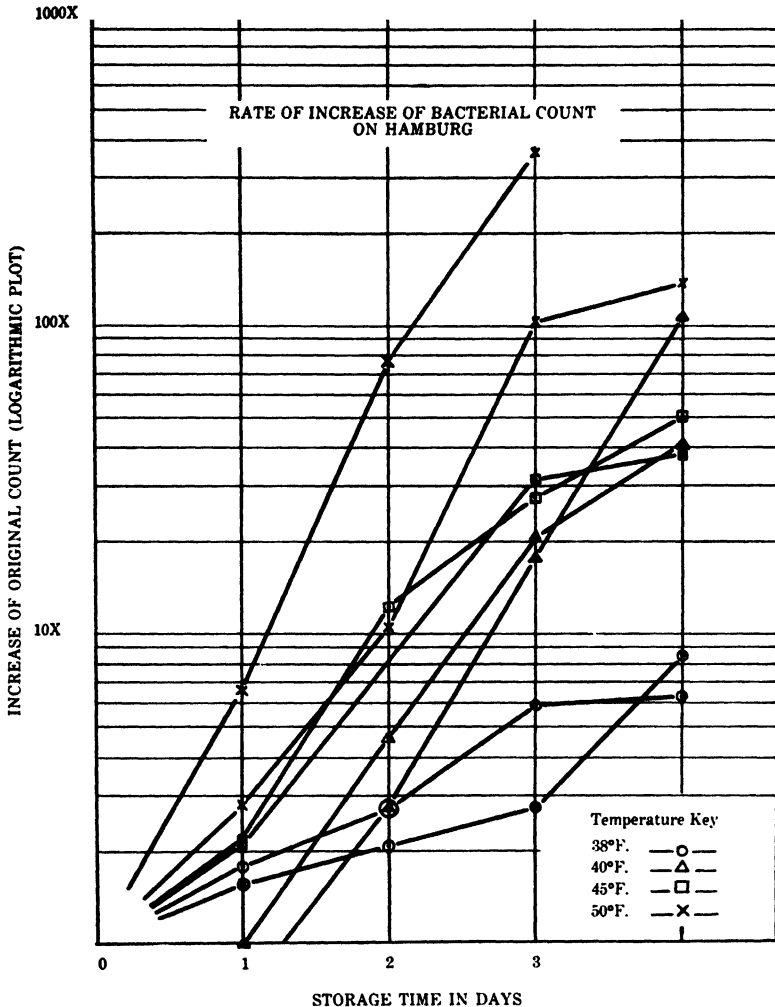


FIG. 2. Rate of increase of bacterial count on hamburger.

These experiments, severe from the standpoint of time interval and the choice of test foods, give some indication of the ranges of physical factors which may be combined to give satisfactory storage for this group of foods.

The results indicate the desirability of low storage temperatures and high humidity conditions combined with moderate air velocities.

FREEZING AND COLD STORAGE OF HERRING

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The supply of British herring is markedly seasonal as regards both quantity and quality. In both these respects there is, so to speak, a feast for one half of the year and a famine for the other. From May till November herrings are caught in enormous numbers, shoaling close round the shores of Britain; from November till May, on the other hand, comparatively few are caught. Herrings feed mainly on zooplankton, which is abundant in early summer, and so replenish their stocks of fat, depleted during the previous winter when their food supply was scarce and during the autumn or spring when they were spawning. The net result of this seasonal variation in the supply of food and of the incidence of maturation and spawning is that in summer and autumn (May to November) herrings are of better quality than in winter and spring. The fat-content of the fish is taken as a criterion of quality, since it is generally agreed that fat fish are in every way more palatable than lean fish. The extent to which the fat-content fluctuates seasonally is astonishing. Quite commonly it rises from about two per cent in the spring to 20 per cent and more in the early summer, the bulk of the rise occurring during about one month's voracious feeding, according to the findings of Lovern and Wood.

The problem of storage of the British herring is therefore to preserve fish of good quality, caught in the summer and autumn, for consumption during the winter and spring, i.e., preservation for some six months. For centuries past, this problem has been solved by curing herrings with salt, but new methods are being called for in the face of diminished demand for this product. Thus, the practice of canning herring in various ways has grown greatly in recent years. On the other hand, freezing and cold storage is little used.

A small quantity of herring is annually frozen in air and stored at temperatures around $-10^{\circ}\text{C}.$ ($14^{\circ}\text{F}.$), and a considerably larger quantity is kippered, frozen in boxes in air at the temperatures just mentioned, and stored for periods up to four months. In both cases the product is inferior to the fresh fish as a result of various changes during freezing and storage. Recent research, however, at the Torry Research Station, Aberdeen, has shown that, under the right conditions, freezing and cold storage involve less alteration than any other

method of preservation and that fish may be kept for several months in a condition almost indistinguishable from that of fresh fish.

The general principles of the successful freezing and cold storage of fish were worked out at the Torry Research Station in the first place for white fish, such as haddock and cod. Storage at about $-10^{\circ}\text{C}.$, the customary commercial temperature, inhibits bacterial spoilage but does not inhibit various forms of alteration, such as development of cold-storage odor and flavor, formation of "drip," and denaturation of proteins which render the fish in a few weeks less palatable and less suitable for smoke-curing than fresh fish. On the other hand, if the following procedure was adopted, the fish retained the flavor and texture of the fresh fish for periods up to six months, and was also entirely suitable for smoke-curing:

1. Freeze the fish rapidly, e.g., in circulating sodium chloride brine at $-20^{\circ}\text{C}.$ ($-4^{\circ}\text{F}.$).
2. Glaze the frozen fish.
3. Store the glazed fish at a low temperature, e.g., -20 to $-30^{\circ}\text{C}.$ (-4 to $-22^{\circ}\text{F}.$) as recommended by Lumley (1935-36) and Reay (1933, 1934, and 1930-36).

White fish, such as haddock, however, has a low fat-content—less than one per cent—while herrings of the sort that might most usefully be stored, have a fat-content anywhere between five and 25 per cent, the bulk of the fat lying just beneath the skin where access of oxygen is easy. In the case of meat of various kinds the preservation of fat is the limiting factor in successful cold storage, for it is more difficult to stop those processes generally included under the term "rancidification" than any other sort of change. In the case of fish, likewise, it has been found that fatty fish like the herring require special precautions and conditions to prevent rancidification that are not necessary in the case of white fish as shown by the work of Banks (1935-36, 1937).

At the Torry Research Station summer herrings were frozen singly in air at $-28^{\circ}\text{C}.$ ($-18.4^{\circ}\text{F}.$) and in sodium chloride brine at $-20^{\circ}\text{C}.$ ($-4^{\circ}\text{F}.$) and stored, with and without a glaze, at temperatures ranging from -10 to $-28^{\circ}\text{C}.$ (14 to $-18.4^{\circ}\text{F}.$) for periods up to six months. The extent of rancidification was judged by tasting the subcutaneous fat—more particularly in the lateral brown band after cooking—and by estimation of the peroxide in and application of the Kreis test to the subcutaneous fat. There was no close quantitative agreement between the increase in rancid flavor and the increase in peroxide or Kreis value; but sufficient parallelism was always found to make it clear that chemical tests may be used as a practical guide to cold-storage procedure.

The quantitative results of these studies show clearly that even over such a low range of temperature as -20 to -28°C . the lowering of temperature has a marked influence upon rancidification; brine-frozen fish, whether glazed or not, become rancid more rapidly than air-frozen fish; and glazing retards rancidification (Fig. 1).

These results suggested experiments *in vitro* with pure herring-oil, when it was again shown that the lowering of temperature retards oxidation (Fig. 2). Moreover, at temperatures between -20 and -28°C . the atmospheric oxidation of the oil was comparatively slow, slower

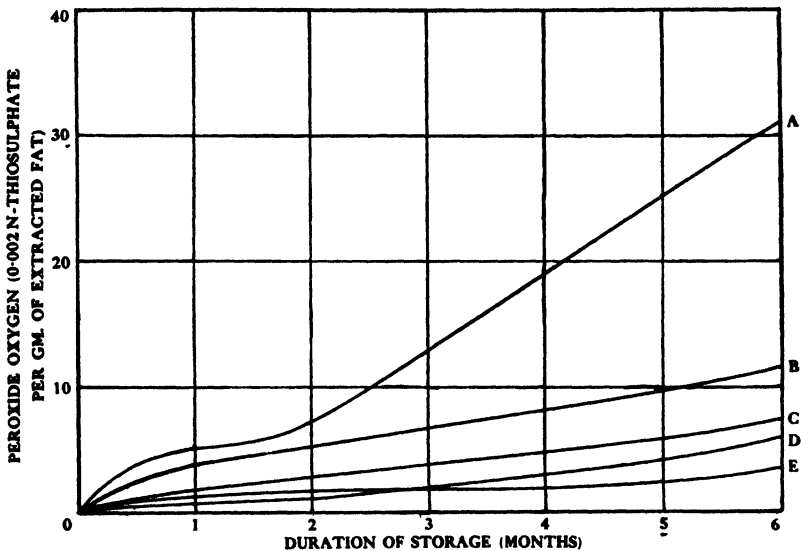


FIG. 1. Peroxide formation in fat of cold stored herring.

Curve A. Brine frozen, unglazed, stored at -20°C .

Curve B. Air frozen, glazed, stored at -20°C .

Curve C. Air frozen, unglazed, stored at -28°C .

Curve D. Brine frozen, glazed, stored at -28°C .

Curve E. Air frozen, glazed, stored at -28°C .

than the results obtained with whole fish might lead one to expect. This is more clearly realized if it is noted that the oil already contained some peroxide at the commencement of the experiment. When, however, minced herring's muscle was mixed with the pure oil, the rate of oxidation was greatly increased; while if the minced muscle had been previously heated for half an hour in boiling water, oxidation was only slightly stimulated. These results have been taken as evidence that the oxidation of herring-oil is catalyzed by an oxidative enzyme present in the flesh of the fish. It was also found that the addition of small amounts of sodium chloride to a system containing enzyme and herring-oil resulted in increased oxidation. Sodium chloride,

therefore, in certain concentrations activates the enzymic oxidation.

These experiments *in vitro* throw light on the results of the tests on the storage of summer herrings. The more rapid rancidification of the brine-frozen fish may be explained by the activating influence of sodium chloride derived from the freezing medium. Much of this is washed off during glazing, but what remains has a definitely noticeable influence. The effect of glazing is best considered by comparing

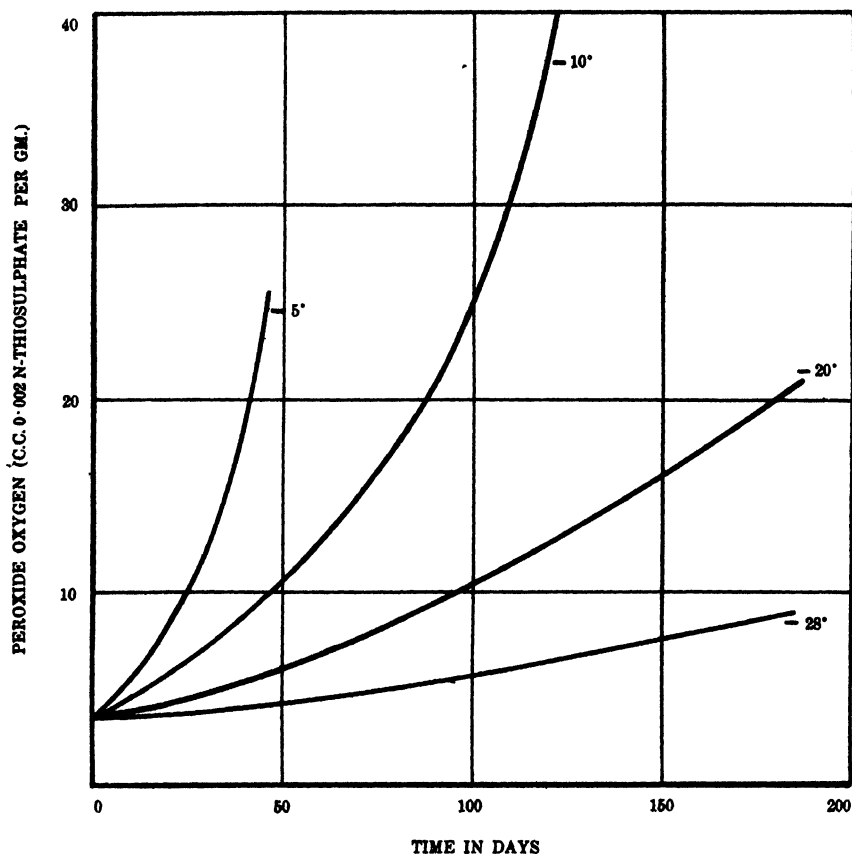


FIG. 2. Peroxide formation in cold stored herring oil.

glazed and unglazed fish frozen in air, the complication of added sodium chloride then being absent. It is often stated that glazing exercises its inhibiting influence by diminishing the access of atmospheric oxygen. Glazing, however, also prevents desiccation in the superficial layers of the fish, and in the light of the "enzyme" hypothesis it is possible that the more rapid rancidification in an unglazed fish is, to some extent, an effect of desiccation.

To revert to the practical problem of storing summer and autumn herring over the winter and spring, experiment has shown that if the fish are frozen rapidly and free from contact with salt (whether by freezing in air or by some method of indirect contact with brine) and are then glazed and held at $-28^{\circ}\text{C}.$, they can be successfully stored for six months. If the fish are frozen in brine, carefully washed and glazed, their storage life at $-28^{\circ}\text{C}.$ is only four months.¹

In conclusion, brief mention should be made of another possible way of cold-storing herring over a period of months. In Britain herring are consumed to a great extent in the form of kippers, and as has been stated, herring rapidly frozen and stored at $-28^{\circ}\text{C}.$ for six months can be made into good kippers. Recent experiments, however, have shown that storing boxes of kippers at $-28^{\circ}\text{C}.$ yields a product, for periods up to six months, that compares favorably with freshly made kippers.

Storing the smoked fish might prove a more flexible method than freezing, storing, and then smoking the fish. Experimental side-by-side tests of these alternatives are in progress.

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¹ These figures do not apply to lean winter fish in which rancidification is slower.

SOME RECENT ADVANCES IN DAIRY TECHNOLOGY

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Although the history of domestication of cattle and use of cow's milk goes far back into antiquity, practically all noteworthy progress in application of science to the art of dairying has been achieved in comparatively recent times. Market milk did not become an important commodity in the United States until the nineteenth century.

HISTORICAL REVIEW

The first significant contribution to dairy technology in this country was a sociological one, for it was concerned with sanitary conditions in the industry. In 1835 an ardent temperance worker, Robert M. Hartley, made the discovery that many or most of the cows in New York City were fed on distillery mash, much to the detriment of their health and the quality of the milk. In 1842 Mr. Hartley published a book on this subject entitled, "An Historical, Scientific and Practical Essay on Milk," which led to various investigations of this so-called "swill" milk, resulted in the introduction of country milk into the city, and brought about many improvements in hygienic conditions at the dairies.

The second noteworthy contribution to dairy technology both chronologically and cogently, was the invention of the first successful process for concentration and preservation of milk. In 1856, after five years of experimentation, a patent for a method of condensing milk in a vacuum was issued to the American inventor, Gail Borden, whose condensed milk appeared on the market a few years later and attained considerable vogue during the Civil War. The first condensed milk was unsweetened, but since its keeping qualities were improved by the admixture of sucrose, the addition of cane sugar became the routine procedure for this product. A patent on a process for evaporating milk without the addition of sugar was granted to John B. Meyenberg in 1884.

The invention of the cream separator in 1878 by the Swedish engineer, Carl Gustav Patrik De Laval, and the perfection of a test for ascertaining the amount of fat in milk and cream by Stephen M. Babcock in 1890, were outstanding achievements in dairy technology prior

to the twentieth century. Another important episode was the invention of the glass milk bottle, devised and patented by Dr. Harvey D. Thatcher in 1884. During this period there were also a number of advances in the science and art of refrigeration which were applicable to the growing dairy industry.

Although Nicolas Appert, the inventor of canning, attempted to dry milk in 1810, and a patent for the dessication of milk was issued in England to Grimwade in 1855, the first successful powdered-milk product was malted milk—a processed blend of milk, whole wheat, and barley malt—patented in 1883 by William Horlick and placed on the market in 1887. Samuel R. Percy had patented a spray method for making dry milk in 1872, but the real development of the powdered milks came after the turn of the century, when the Just roller method was patented in 1902 and the Merrell-Gere spray method in 1906. Between 1900 and 1919 various other procedures for the dessication of milk were also patented, but the spray method is the one now most widely employed for the approximately 300,000,000 pounds produced annually in this country.

DEVELOPMENT OF PASTEURIZATION

Perhaps the most significant of all the earlier contributions to dairy technology was the development of the process of pasteurization. The application of heat to the conservation of foods had been studied in 1765 by the Italian biologist, Lazzaro Spallanzani, and had been investigated by the Swedish chemist, Carl Wilhelm Scheele, in 1782, and by Nicolas Appert in France; but the credit for the practical application and scientific explanation of these procedures belongs to Louis Pasteur, whose name is perpetuated in the term “pasteurization.”

Between 1857 and 1862 Pasteur demonstrated that the souring of milk was due to microbes believed to have come from the air. From 1860 to 1864 the great French chemist studied diseases of wines and revealed that heating would arrest adverse fermentation. In 1870 he investigated beer, developing a method of heating, or pasteurization, of this product.

Although the condensed milk invented by Gail Borden was submitted to a process virtually equivalent to pasteurization, heating was not applied to market milk until about 1880, when pasteurizing machinery was developed in Germany, and later in Denmark. By 1885 market milk was being pasteurized regularly in Copenhagen and Stockholm although for commercial rather than hygienic reasons.

In 1890 pasteurizing machinery from Denmark was introduced into the United States although its employment was limited for many

years. About this same time (1890) the first bacterial analyses of a city milk supply were made by the late Professor William Thompson Sedgwick of the Massachusetts Institute of Technology, and certified milk was originated in 1893 by Dr. Henry L. Coit of Newark, New Jersey.

In this decade a number of progressive physicians, including Drs. A. Caillé, Abraham Jacobi, Henry Koplik, and Rowland G. Freeman, all of New York, advocated the heating of all milk used in infant feeding, but the greatest impetus to the movement for safe milk came from the activities of the philanthropist, Nathan Straus, who established in 1893 the first of his numerous milk stations in New York City for the furnishing of pasteurized milk to the poor.

The invention of a holding method for pasteurization in 1895 by Professor H. L. Russell, the standardization of the method about 1900 as the heating of milk at 62.8°C. (145°F.) for 30 minutes, the requirement of general pasteurization in Chicago in 1909, and a similar requirement in New York City in 1914, all gave further incentive to the more general pasteurization of market-milk supplies, an impetus that gained increased momentum from numerous epidemics of milk-borne diseases traced to contaminated raw milks.

Although the first evidence that polluted milk might spread disease, in this case typhoid fever, was adduced by Dr. Michael Taylor at Penrith, England, in 1857, and this same physician demonstrated ten years later that scarlet fever could be carried by milk, the foremost epidemiological investigation of a milk-borne outbreak was the work of Professor Sedgwick, who in 1892 traced a typhoid epidemic in Springfield, Massachusetts, to an unsuspected milk supply.

As pasteurization became more widely adopted, pasteurizing machinery was developed and perfected. A notable study published in 1925 by the United States Public Health Service showed that a temperature of 142°F. for 30 minutes was adequate to produce bacterial safety, while subsequent studies by engineers of the Public Health Service indicated ways and means to accomplish greater efficiency in the pasteurization process, especially by elimination of various defects in the existing machinery.

Today it is estimated on reliable authority that about 88 per cent of our market-milk supplies in cities of 10,000 or more population is pasteurized. In many of our large cities nearly 100 per cent pasteurization has been achieved, but the picture is less roseate in the smaller communities of this country. In places having populations between 1,000 and 10,000 only about 39 per cent of the milk is now pasteurized, the proportion usually growing less and less as the population decreases.

From the standpoint of modern dairy technology, efficient pasteurization of all market milk is an essential sanitary measure. As expressed so vigorously and satisfactorily in a recent (September, 1937) editorial in the *American Journal of Public Health*, "Raw milk should no longer be permitted to impede public health progress."

NUTRITIVE VALUE OF MILK AND DAIRY PRODUCTS

Since dairy technology is concerned not only with the mechanics, physics, and chemistry of the art and science of milk production, preservation, and distribution but also with applied biology and biochemistry, the contribution and place of milk and dairy products in the newer knowledge of nutrition deserves passing comment.

Many of the clues to the modern science of dietetics were, in fact, derived from studies on milk, a product recognized from time immemorial as an indispensable food for growth and health. In 1881, for instance, the German investigator, Lunin, observed that laboratory animals lived for months on milk but succumbed quickly when fed a combination of the purified constituents of milk. His conclusion that milk must contain other substances essential to nutrition was confirmed ten years later by Socin, another German research worker.

In 1906, about a decade after Eijkman's famous report on beriberi, Hopkins in England noticed that experimental animals failed to thrive on purified diets comprised of casein, lard, starch, cane sugar, and mineral salts, but that they grew satisfactorily when small amounts of milk were added to their diets. Like Lunin, Hopkins concluded that milk must contain substances necessary for growth and health.

The simultaneous discovery in 1913 of the fat-soluble vitamin by McCollum and Davis and by Osborne and Mendel resulted in each instance from experiments on butterfat, while the water-soluble vitamin reported by McCollum and Davis in 1915 was found in a milk constituent. Vitamin D was differentiated from vitamin A in 1921 as the result of work on butterfat and cod liver oil.

At the turn of the century, milk was regarded by chemists merely as a well-balanced combination of fat, complete proteins, carbohydrate, and minerals dissolved or suspended in water. Modern chemists are, however, familiar with approximately 100 different constituents of milk, including 11 glyceryl esters of fatty acids in its butterfat; 22 amino acids in its three proteins; 25 minerals, of which calcium is the leading representative; one carbohydrate (lactose) in two forms; at least eight vitamins; several provitamins, such as carotene and cholesterol; eight enzymes; and various miscellaneous substances, such as

lecithin, lactochrome, and other non-protein nitrogenous substances, citric acid, phosphorus compounds, lactic acid, and dissolved gases.

There are, moreover, substances in milk as yet undiscovered, or unidentified, such as the growth-promoting factor associated with milk produced in the summer by cows fed on rapidly growing green plants. As Professor Henry C. Sherman has indicated, milk may be one of those "wholes" which, while chemically a mixture, is in the philosophical sense a sort of synthesis whose significance is greater than a mere summation of its parts. Certainly no chemist can as yet duplicate milk in the laboratory as it comes naturally from the cow.

MORE EFFICIENT MACHINERY AND EQUIPMENT

The more recent developments in dairy technology have been concerned with new and improved machinery and equipment for the production, processing, and handling of milk and its products; with new methods for promoting and maintaining the cleanliness, purity, and safety of milk; and with new procedures for increasing and studying the nutritive qualities of this lacteal product, which already has earned the apt characterization of "our most nearly perfect food."

In the field of dairy equipment the refrigerated railroad tank car, which came into use in 1924, should be mentioned as should also the refrigerated automobile truck for milk transportation. Recently, stream-lined tanks cars have been introduced in conformity to modern trends in railroad construction. A fleet of 60 of these cars was put in operation by one company early in 1936.

New metals which are less corrosive than those formerly employed and which are less reactive with milk have been developed for use in dairy equipment. Among these are stainless steel and pure aluminum, which have been adopted for dairy purposes only within the past few years. Washing powders and chemical sterilizers, such as chlorine compounds, have likewise been developed, although rigid cleanliness in the operation of all dairy equipment is, as always, a chief desideratum in efficient operation.

Another new development has been the paper milk bottle, first used for regular delivery service of market milk in 1929, but of relatively little commercial importance until 1935, when about 125,000,000 of these containers were in use. Today, there are half a dozen manufacturers of paper milk bottles, one of whom is now said to be producing at the rate of 2,000,000 a week.

One firm in New York City is reported to be using these paper containers at the rate of nearly 100,000,000 a year for sales in stores and for deliveries to schools. Since the paper milk bottle possesses

many advantages, and since its only apparent consumer disadvantage is its lack of transparency and its inability to reveal the cream line of the milk, a decided increase in the use of these convenient sanitary containers may be expected in the future.

One of the most interesting contributions to the machinery involved in recent dairy technology is the rotary milking machine, or Rotolactor, conceived by Henry W. Jeffers of the Walker-Gordon Laboratories of The Borden Company. This machine, designed by engineers of the De Laval Separator Company, was first put in operation at the Walker-Gordon farm at Plainsboro, New Jersey, in November, 1930, when it was started in motion by the late Thomas A. Edison.

The Rotolactor consists of a slowly revolving, circular platform, having spaces for 50 cows which are automatically stanchioned, washed, dried, and then milked by means of milking machines in the short space of 12½ minutes each. The cows are then automatically unstanchioned and allowed to return through passageways to their barns; while the milk, which has been collected in pyrex glass jars, is weighed, dumped, and piped through sanitary pipes to a bottling room where it is bottled by machine.

Aside from the sanitary features of this rotary milking machine, it has the economic advantage of centralizing milking procedures and reducing the manual labor of milking. At present there is only one Rotolactor, but a similar mechanical principle has been adopted in the so-called milking parlor in which cows are brought to a central place for milking under hygienic conditions. Many of these milking parlors have been established in recent years at leading dairies.

The application of modern engineering to the dairy industry is emphasized in a number of extensive new city milk plants that have been opened recently or are in process of construction in such cities as San Francisco, St. Louis, San Antonio, and New York, to mention only a few. From reception of the raw milk, usually in trailer-mounted tank trucks, through the consecutive operations of weighing, cooling, storage, filtration, irradiation, pasteurization, cooling, bottling, packing and icing, and delivery, the latest mechanical devices and equipment are employed. Huge can washers and bottle washers, handling 120 bottles per minute, take care of these sanitary needs, while refrigeration, ventilation and other accessories are provided by the most modern equipment. To see dairy technology at its best, a visit should be paid to one of these new milk plants, which often are and always should be meccas for students and scientists desiring intellectual stimulation, as well as for sightseers and tourists.

Developments in the field of milk sanitation have kept pace with mechanical developments in the dairy industry, although full applica-

tion is not always given to the knowledge, especially in rural sections. There is still an average of about 40 milk-borne disease epidemics a year in the United States, owing chiefly to contaminated raw milk supplies in the smaller communities.

Recent advances in dairy hygiene have been the recognition of brucellosis (undulant fever) and the inauguration of a national campaign for the eradication of this disease from dairy cattle. Bovine tuberculosis is still a problem in many areas, but the dangers of both this disease and brucellosis can be completely obviated by universal pasteurization.

There has been in recent years a recurrence of interest in high-temperature, short-time pasteurization. New equipment has been designed for the effective heating of milk to $71.1^{\circ}\text{C}.$ ($160^{\circ}\text{F}.$) for 15 seconds, a process which will give the same degree of safety as the older holding method. Because of economy in space and time, this method deserves wider use and greater sanction by health departments, many of which already have given their approval.

DEVELOPMENT OF IRRADIATION OF MILK

Another distinctive advance in dairy science has been the development of apparatus and a technique for the irradiation of milk, a process that enhances the natural antirachitic properties of milk by converting the cholesterol in the butterfat, and to a slight degree in the protein, into one of the various forms of vitamin D. The antirachitic potency of milk may also be increased by scientific feeding of irradiated yeast to producing cattle and by the direct addition to the milk of one of the various concentrates of vitamin D.

Irradiation of milk is an outgrowth of the investigations of the late Dr. Alfred F. Hess of New York and Professor Harry Steenbock of Wisconsin, who announced independently in 1924 that antirachitic properties could be imparted to certain foods by irradiation with ultra-violet light.

The first commercially distributed, fluid, vitamin D milk was, however, a certified milk increased in antirachitic properties by the yeast-feeding method and put on the market by the Walker-Gordon Laboratories in the summer of 1931, following a clinical report on the efficacy of this metabolized milk presented by Dr. A. F. Hess at the annual meeting of the American Medical Association. Late in 1932 the first irradiated, fluid, vitamin D milk appeared on the market in Detroit, while irradiated evaporated milk was first manufactured in 1934. In the meantime, fluid milk modified with a concentrate of vitamin D had also made its appearance.

No one seems to know precisely how much vitamin D milk of all types is now distributed but a fair estimate is about 3,000,000 quarts a day, of which about 75 per cent is in the form of irradiated evaporated milk. Numerous clinical and laboratory investigations have been conducted on these milks, so that there is now a vast scientific literature on the subject, so extensive and involved, in fact, as to be almost bewildering.

It is the consensus of medical and scientific opinion, however, that vitamin D milks of proper potency are of distinct value in the prophylaxis and cure of infantile rickets, and that this development in modern dairy technology has been the most important contribution to dairy science since pasteurization. The more general use of vitamin D milks, which also provide the calcium and phosphorus required for antirachitic efficiency, would tend further to reduce the incidence of rickets, a disease that is still one of our important public-health problems.

ADDITIONAL STUDIES IN CONNECTION WITH MILK

Other vitamins in milk have also enlisted the attention of scientific investigators. Recent studies have shown that freshly produced raw milk is an excellent source of vitamin C, and that this antiscorbutic factor can be retained in pasteurized milk by protecting it from exposure to light, oxidizing influences, and destructive enzymes. Instead of losing from 20 to 50 per cent of vitamin C in the pasteurization process, this important food factor can be preserved in virtually full potency by proper methods, although in infant feeding vitamin C supplements may still be necessary as a factor of safety.

The vitamin B complex in milk has been studied. As this vitamin is broken down into half a dozen different components, milk is found to be excellently provided with most of them. A method for isolating one of these fractions, riboflavin, or lactoflavin, and also of measuring it with "black light" has been devised recently by Supplee.

Factors in the digestibility of milk have provoked the interest of a number of investigators, particularly with reference to so-called soft-curd milks. Not only have studies been made on the factors influencing the curd tension of the milks of various breeds of cattle and of individual cows within the different breeds, but a more or less practical method for altering the curd of average fluid milk has been devised. The curd of milk may also be softened by homogenization and by the processing to which it is subjected in the manufacture of evaporated, condensed, and dried milks.

The quick freezing of milk and dairy products for their better preservation, storage, and transportation has received attention, and

new methods have been worked out. Rapid freezing of breast milk with dry ice by a process invented by Platt in 1935 is now being carried out at a number of mothers' milk bureaus in different cities, thus providing a reserve of breast milk to care for its fluctuating supply and demand.

Much attention has been given in recent years to the technology of cattle feeding in order to provide and preserve feeds that will yield the optimum nutriment in market milk and by their continuous use throughout the year will furnish milk of uniform quality, rich in those vitamins that are favorably influenced by the diet of the cow. Iodine can also be increased in milk by proper feeding although this is the only mineral in milk that is materially affected by the cow's dietary regimen.

It is obvious that much has been accomplished in recent years, but many important problems remain to be solved. Some of them are economic and sociological as well as scientific, for milk consumption in this country is far below the standard agreed upon as most desirable from the standpoint of our national vitality. The increases in the use of milk by our people that have occurred since 1900 have been due chiefly to greater confidence in the safety of this nourishing food, to its greater availability in attractive form, to a better understanding of the economy of its nutritive virtues, and to higher standards of living.

In most of these matters, modern dairy technology has been a significant factor. In the future, it should be even more significant.

APPARATUS FOR MEASUREMENTS OF CHEWING RESISTANCE OR TENDERNESS OF FOODSTUFFS

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INTRODUCTION

One of the important problems of food technology is the tendering of foodstuffs, such as meat and fruit. The conception of tenderness is highly complicated since it includes physical, chemical, and physiological elements. It is not possible at present to express tenderness as clearly and simply as, for example, viscosity is determined in physics. Nevertheless, several attempts have already been made to measure the tenderness of foodstuffs. Most of them are based on the principle of a penetrometer and depend on the measurement of the force which must be applied for a certain deformation, tearing, or squeezing of a sample of food.

An apparatus has been developed which allows recording of the force as a function of the resulting deformation and determination of the total energy utilized for this deformation. We suppose that the values obtained in this way may reproduce the real chewing resistance of foodstuffs and may serve as a measure of their tenderness.

DESCRIPTION OF APPARATUS

When being chewed, portions of food are squeezed between the molar teeth (grinders) and in this way reduced in size. As a substitute for teeth, we have chosen two wedges with rounded points (Fig. 1). Sharp knives are too far removed in their manner of action to act as substitutes for teeth. It is also difficult to get comparable results when using knives, for the degree of their sharpness is not easy to reproduce.

In foodstuffs of fibrous structure, such as meat, the greatest resistance results if the fibers lie in a course vertical to the axle of the wedges (Fig. 1, A and B). The deformation of the sample after the wedges have approached nearer to each other is shown (Fig. 1, B). With decreasing distance a between the wedges the squeezing force P increases and at the same time tensile forces (tractions) arise in the fibers. If these tractions become strong enough, the fibers are gradually torn. This process can be followed by recording a curve $P=f(a)$.

In the scheme of the apparatus a lever H balanced by weights G_s and G_w turns around the axle O_1 (Fig. 2). The lever K is attached to lever H. If the lever H is turned contrary to the direction of the hands of a watch, the upper wedge approaches the lower one, which is fixed on the frame, and squeezes a sample put between the wedges.

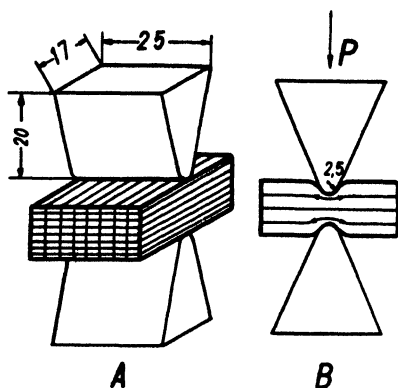


FIG. 1. Squeezing of a fibrous sample with rounded wedges.

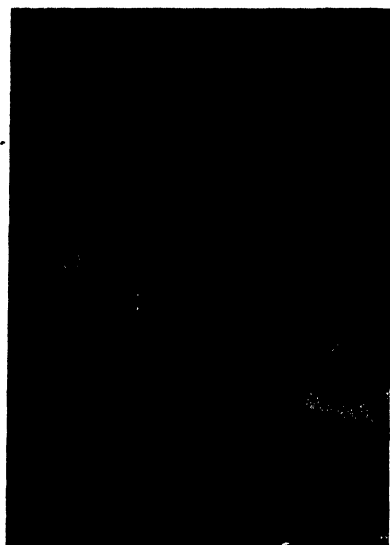


FIG. 3

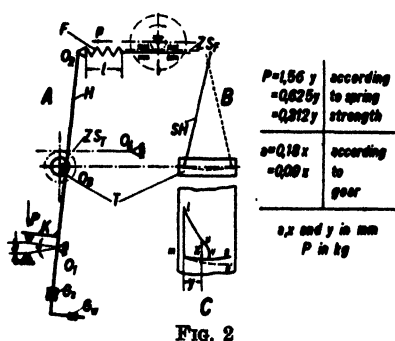


FIG. 2



FIG. 4

The distance a is generally less than 10 mm., the length of the lever K amounts to 80 mm., so that the movement of the wedges and the direction of the squeezing force P can be assumed as vertical. Since the length of the lever H is 400 mm., $P = 5 p$ follows. The force p is transferred to the lever H with the aid of a spring F

through the rack ZS_F by means of a cogwheel. The contraction Δl of the spring is a measure for the force p , and the angle of rotation S of the lever H is a measure for the distance a . The correlation of these two magnitudes can be represented by a curve drawn on the drum T of the recording device.

The lever SH of the recording device (Fig. 3) turns around point O . It moves only when the spring length is changed. The drum, on the other hand, turns only with the rotation of the lever H , that is to say, with the change of the distance a between the squeezing wedges. The rotation of the drum is effected by the rack ZS_T (Fig. 2) tied to the cogwheel of the drum, the end O_4 of which is joined to the frame.

If there is no sample between the wedges, the lever H_0 remains motionless if the wedges approach each other and the pencil at the end of the lever draws a straight line lm , which runs parallel with the border line of the drum (Fig. 2, c). After the two wedges come in contact, the drum comes to a standstill. If the movement of the squeezing drive is continued, the spring F shortens and the lever H_0 describes an arc $m-n$.

When squeezing a sample, the two movements are executed at the same time and, in this case, the pencil records a curve $l-u-v$. The ordinates y represent the squeezing force P in kg., so that $P = 1.56 y$, or $P = .625 y$, or $P = .312 y$ (y in mm.), according to spring strength. The distance a is calculated from the abscissae x ; $a = .18 x$, or $a = .09 x$, depending on the gear ratio between the drum and the rack ZS_T .

As regards the accuracy of the measurement, the greatest error in measuring the force is about 150 grams, and in measuring the distance a , about .1 mm. The forces which arise from squeezing lie usually between 10 and 120 kilograms.

In consequence of the deformation of the frame, a different arc $m-n'$ results from strong springs than from weak springs (Fig. 2, c). The difference $n-n'$ at the greatest ordinates corresponds to an error of .4 mm. If, however, x is always measured from the arc, the error remains .1 mm. for all springs. Additional parts to this apparatus have been constructed to enable fruit tests.

EXPERIMENTAL DATA

The power scale for the curves a to c is 1 mm. \equiv .312 kg. and for the curve d , 1 mm. \equiv 1.56 kg. (Fig. 5). For these experiments strips eight mm. thick have been cut out of boiled meat, following the course of fibers. A double knife (Fig. 3 on table) was used for cutting. Then the strips were cut to a width of 25 mm., which corresponds to the

unbroken fibers being chewed considerably influences the subjective judgment of tenderness.

It is likewise apparent that the fluctuations of the curves are much greater than the recorded accuracy of the squeezing force, the error hereby having been estimated as .15 kg. The reason for these great fluctuations is that meat is not at all homogeneous; nevertheless, the fluctuations could hardly be identified at subjective examination.

The upper group of five curves (Fig. 5 c) was taken from test pieces of beef; the two lower curves refer to veal. Veal and beef were boiled; both types of meat were found to be tender by subjective tests, but in any case the impression was gained that beef was not quite so tender as veal. Probably this impression may be principally due to the remainder of stronger fibers which the upper five curves show. The absence of the second increase of squeezing force in the case of veal proves that veal has a more homogeneous structure than beef.

All the curves (Fig. 5, d) refer to one single piece of beef which subjectively has been marked as tough. The lower curves have been traced, using wedges, the upper ones using denture. First, the figure shows that with denture the resistance of a test piece is about twice as high as with wedges. Second, the upper curves are showing a second increase which is entirely absent at the lower curves. We can explain these results only by the fact that the meat was confined and compressed within the denture to a considerable extent, as it is impossible to liberate some excess portion of the sample when the teeth approach closely. The meat sticks between the teeth surfaces, owing to the irregular profile, thus producing the second increase mentioned before. If this explanation is right, one might conclude that wedges are more suitable for measurements than the denture; since the use of wedges enables one to observe and determine definite structural distinctions in fibers which are not homogeneous.

Such experiments were made also with bacon, and they demonstrated again that the resistance of test pieces when using the denture is twice as high as with the use of wedges. This fact indicates again the desirability of substituting the denture with wedges.

These first experiments are certainly not yet sufficient to make a definite statement. We intend to continue them more systematically, hoping that the apparatus developed will enable us to measure correctly the tenderness of several foodstuffs.

DISPOSAL OF FOOD-PLANT WASTES

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Among the many problems confronting food manufacturers that of utilization or inoffensive disposal of solid and liquid wastes is becoming increasingly important. Since many food-processing wastes are much stronger than equal volumes of ordinary domestic sewage, and also frequently differ from sewage in chemical and other characteristics, discharge of these wastes untreated into public sewerage systems has caused difficulty at municipal sewage treatment works. Such wastes emptied directly into water-courses have given rise to an increasing demand by the public for proper treatment of food-plant wastes.

Processes employed in the treatment of food-plant wastes to remove unstable organic substances may in general be classed as mechanical, chemical, biological, or combinations of these processes. These various processes are illustrated by the developments in Wisconsin in the treatment and disposal of vegetable cannery wastes.

During 1925 a coöperative waste treatment research program was proposed to the Wisconsin Cannery Association by the Wisconsin State Board of Health. It was pointed out that satisfactory methods of treating cannery wastes had not yet been developed, and that, while this was the problem of the industry, the state would coöperate in developing such methods. Since the pea-cannery wastes offer the major problem in Wisconsin, preliminary activities were confined to the development of practical and economical means of treating them.

Wastes receiving treatment included blancher effluent, resembling pea soup; produce washings before and after blanching; and floor and equipment washings. Silage juice produced by fermentation of stacked pea vines was disposed of by soil absorption at viner stations on nearby farms. Water used to cool the canned peas was by-passed and used to dilute the treated effluent. Domestic sewage from toilets and lavatories was disposed of separately by means of a septic tank. The volume of waste amounted to about one gallon for each No. 2 size can of peas packed.

Results of the preliminary studies were used in the design and installation of waste-treatment plants during 1927 at pea canneries where very unsatisfactory conditions existed. The plants were of two

general types, classified as continuous-flow and fill-and-draw systems, depending on the method of handling the wastes. Operating experiences with seven treatment plants indicated that the fill-and-draw system gave more satisfactory results, owing largely to more exact chemical application and greater ease in the control of treatment and better facilities for sludge removal.

Screening of the wastes is the first step in removal of organic substances and is necessary irrespective of the method adopted for fur-

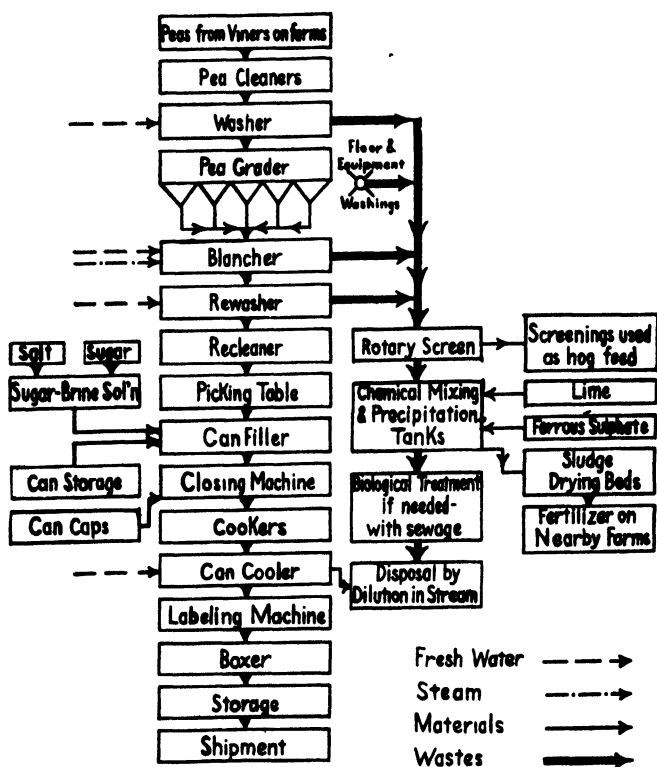


FIG. 1. Flow chart, showing pea-canning process and waste-treatment system.

ther treatment. Experience has indicated that most effective results are obtained with mechanical rotary-screen units having from 20- to 40-mesh wire covering.

Operating results have indicated that by the use of chemical treatment plants the objectionable organic substances in the wastes, as measured by the oxygen demand, were reduced from 50 to 75 per cent by application of $3\frac{1}{4}$ pounds of ferrous sulphate (copperas) and $7\frac{1}{4}$ pounds of lime per 1,000 gallons of pea-canning wastes; $2\frac{1}{4}$ pounds of ferrous sulphate and 6 pounds of lime per 1,000 gallons

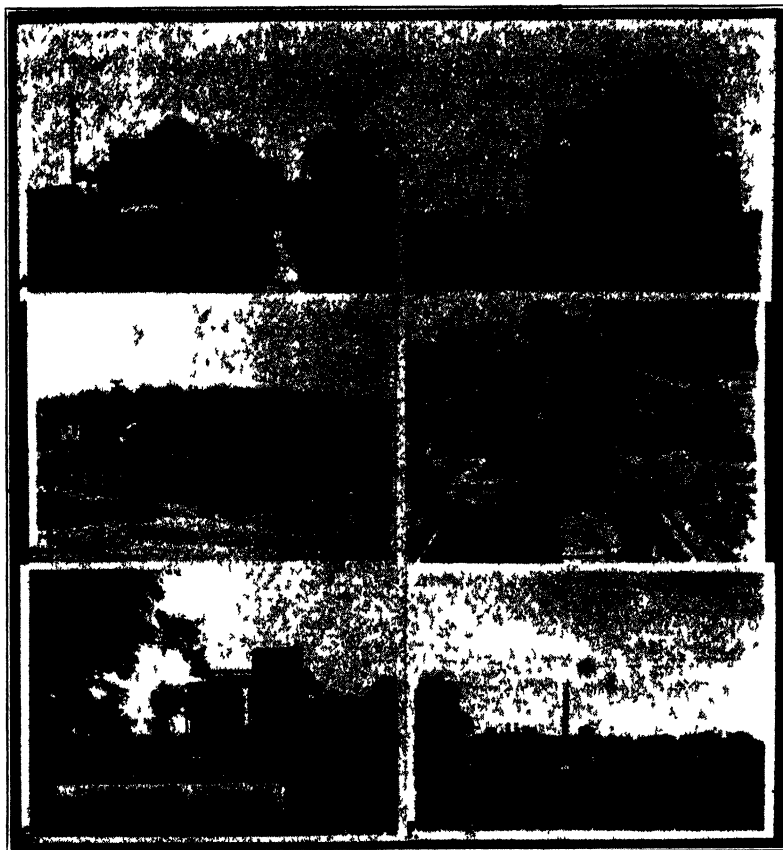


Fig. 2 Food waste disposal systems.

- A. Experimental trickling filter for biological treatment**
- B Typical full scale installation of a trickling filter plant**
- C D Chemical treatment systems to fill and draw type for cannery wastes and**
- E Layout of continuous flow system for cannery wastes**
 - a. Rotary screen**
 - b. Mixing channel for chemicals**
 - c. Settling tank**
 - d. Mechanical equipment for removal of precipitated solids.**
- F. Trickling filters like this are used where a high degree of treatment is essential with food plant wastes.**

of wax-bean wastes, and $3\frac{1}{4}$ pounds of ferrous sulphate and 6 pounds of lime per 1,000 gallons of corn wastes. Experience showed that thorough mixing of the chemicals with the waste and a settling period of at least one hour are required to insure proper treatment; daily removal of the sludge is essential.

Studies of treatment of pea-cannery wastes by use of a trickling filter, both with and without chemical precipitation in advance of

filtration, were started in 1928. The data and observations indicated that by the use of a seeding procedure the treatment efficiency of a trickling filter may be, in large part, established before canning starts.

Results of the trickling-filter studies were used as the basis of a plan for secondary treatment of sewage recently carried out in a Wisconsin municipality, whereby wastes from a cannery (after chemical treatment) are discharged into the dosing tank of the new trickling filter at the city plant. From the results obtained to date this arrangement has been successful in alleviating a very serious pollution problem.



FIG. 8. Solids removed from cannery wastes by chemical treatment are dried on sand beds and utilized on farms as fertilizer.

The discharge of the cannery waste by separate pipe line into the dosing tank of a trickling filter at a municipal plant is unnecessary if an ample detention period is available in the primary tanks, and provided that sufficient filter capacity for the combination of chemically treated cannery wastes and domestic sewage is available. This has been shown by operating results obtained in another municipality.

Studies have also been conducted of complete treatment of cannery wastes along with domestic sewage at activated sludge plants. Ample aeration capacity is essential. Tests indicate no interference with the digestion or breaking down of the sludge produced. In each situation the cannery wastes are given pretreatment by screening and chemical precipitation.

Much work remains to be accomplished both in applying present knowledge and in continuing the research to improve existing, and to develop new, facilities to permit still more efficient and economical utilization or treatment of canning-plant wastes. It has been shown that this work can most advantageously be performed with the support and coöperation of the National Cannery Association and the various state cannery associations.

UTILIZATION OF WHEY IN FOODS¹

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A large portion of the world's whey produced as a by-product in cheese manufacture has always been a waste product. During recent years the manufacture of commercial rennet casein has increased the volume of edible whey and further complicated the problem of disposal. The whey from many modern dairy plants and cheese factories is a liability and an expense to discard. Much of the whey utilized today is added to stock foods, while an extremely small portion of the country's production is condensed or dried for human food.

The nutritive value of whey is high. Only the casein and the fat of milk are removed in cheesemaking. The remaining solids, lactose, salts, and whey proteins are, in the light of the present science of nutrition, assuming a vital significance as foods. There is some experimental evidence which indicates that lactose may be of importance in calcium metabolism. If future research shows lactose to be a dietary necessity in this respect, the utilization of whey in food will be a simple means of providing lactose. Whey is a good source of calcium and phosphorus. It contains also practically all of the milk albumin. Perhaps the largest outlet for whey should be as a constituent of human food. Actually, however, the amount of whey used in food is extremely small. The work of the Bureau of Dairy Industry on the utilization of whey was undertaken in the hope that part of the five-billion-pound surplus could be turned into useful food through the development of new whey products.

Certain obstacles become apparent when one considers seriously the introduction of whey solids in different foods. An approximate analysis of separated fresh and dried whey is as follows:

	Fresh whey pct.	Whey powder pct.
Lactose.....	4.9	73.5
Ash.....	0.6	9.0
Protein.....	0.9	13.5
Water.....	93.6	4.0

¹ Presented before the Conference on Food Technology, Massachusetts Institute of Technology, September 14, 1937.

The whey products described in this paper were developed in the Division of Dairy Research Laboratories by Mr. G. A. Ramsdell and the author, with the assistance of Mr. C. F. Hufnagel.

The high concentration of lactose in whey, coupled with its low solubility in water, limits the quantity which may be used in many recipes. Forced crystallization may be resorted to when the solubility of lactose is exceeded, but under such conditions the sandy effect of the crystals must somehow be covered up.

The salt content of whey becomes very noticeable and in some foods objectionable when the product is used in concentrated form.

The foaming properties of whey protein are well known. Normal whey contains insufficient protein, however, and has too low a viscosity to produce and support a stable foam. If a whey-protein mixture could be cheaply prepared with a relatively low lactose and salt content and the approximate albumin concentration of egg white, it might possibly possess that rare combination of foaming and heat-coagulative properties which make egg white almost unique. Since such a whey-protein product is not yet commercially practical to manufacture, it is not always possible to utilize whey protein in place of egg white where high whipping properties and coagulation under heat are required.

The natural rather insipid taste of whey is not generally pleasing, and therefore, bars the use of whey directly as a beverage. Even when made into punch or mixed with fruit juices it contributes no desirable characteristics to the taste of the product. It acts essentially as a substitute for water or as a purely desirable addition to the drink from a nutritive standpoint.

The problem of the utilization of whey in foods resolves itself to the task of finding new and unique uses for whey solids. If uses can be discovered of such a nature that other products such as skim milk cannot be substituted for whey, a real outlet for whey solids will have been uncovered.

Skim milk, with its casein, has a distinct advantage over whey in many foods. In such cases and in foods in which both serve equally well, skim-milk solids will be chosen in preference to whey solids because at present the former are more readily available and generally lower in cost. Unless or until whey solids can be sold at a price advantage in comparison with skim milk, successful utilization of whey in foods must depend upon its unusual characteristics.

Foods containing whey must rely upon their good flavor and appealing appearance if they are to attain heights of consumption sufficient to yield an important outlet for whey solids. Appeal on the grounds of nutritive qualities and healthful characteristics, while sound, is not an easy way to secure consumer volume.

We have studied the use of whey solids in canned processed foods. Fresh, condensed, or powdered whey was added to many different

products. Some of these foods were not improved by the addition of whey solids, while in others the addition of whey was beneficial.

WHEY SOUPS

Whey solids were successfully used in canned soups. During the manufacture of cream soups difficulty is encountered with the coagulation of casein. Coagulation is influenced by a number of different factors and it cannot be avoided in cream soups.

Experimental work proved that substitution of whey solids for skim-milk solids resulted in a soup which was more easily processed and showed less protein coagulation. The explanation was not hard to find. The whey soups, being casein free, contained less heat-coagulable protein. It was observed that whey protein showed less tendency to coagulate in large hard flocks than did casein.

Many batches of soup were made on a laboratory scale, comparing milk soups and whey soups. Their composition was varied over a wide range. A softer, finer, and less noticeable coagulum developed in the whey products than appeared in the soups containing milk.

Whey solids offer an improvement over milk solids in tomato soup from the standpoint of their lower buffer action. More natural tomato acidity is retained in whey soup; somewhat milder processing may consequently be used. Trouble with casein coagulation during the preparation of the soup is avoided and no neutralization of the tomato acid is necessary. Tomato soup containing whey solids may be boiled and held hot by the consumer without any danger of protein aggregation or lumping.

The approximate composition of some canned whey soups is as follows:

	Whey solids pct.	Butter- fat pct.	Flour pct.	Salt pct.
Celery.....	3.0	3.0	3.3	1.0
Tomato.....	4.5	3.0	2.8	1.0
		Sugar pct.	Vegetable juice and pulp pct.	pH before processing
Celery.....		24	5.99
Tomato.....		1.2	70	4.43

Whey solids in the form of condensed or dry whey would be a valuable addition to home- or restaurant-prepared soups. When milk is not available, the housewife could add greatly to the nutritive value and flavor of her soups through the addition of whey powder. If restaurants were supplied twice weekly with fresh condensed whey

they would find it possible to utilize this product as an inexpensive source of valuable whey solids in soups, gravies, and sauces.

A combination beverage and soup was made by mixing tomato juice and whey. The best formula for this tomato-whey juice was found to be:

Tomato juice.....	65.0%
Fresh whey and whey cream.....	34.6%
Salt.....	0.4%

The reaction varied between pH 4.3 to 4.5. The fresh whey was sweet and contained enough added fat or whey cream to give the finished mixture a butterfat content of about two and one-half per cent. The product was homogenized at 2,500 pounds pressure after heating to 60°C.(140°F.). It was canned and sterilized at once.

Tomato-whey juice contained minute particles of coagulated whey protein and tomato pulp which were not considered objectionable. Separation on storage was slight and the mixture became homogeneous after a simple inversion of the can. The product was excellent as a cold drink. It was also used as a thin tomato soup; and by the addition of flour, a normal cream-style tomato soup resulted. This product was one of the simplest to make and one of the most satisfactory of all the canned goods prepared with whey.

SWEETENED CONDENSED WHEY

The foaming properties of whey are well known but there have been few attempts to utilize this characteristic in the incorporation of whey in foods. Taken at its normal concentration whey is too thin to produce a permanent foam. The Bureau of Dairy Industry developed a method whereby both the foaming properties and the keeping quality of fresh whey could be increased. A sweetened condensed whey was made by condensing, under vacuum, fresh pasteurized whey to which sugar had been added equal in weight to the solids of the whey. This mixture was condensed to approximately 75 per cent total solids and cooled with stirring to cause the lactose to crystallize in a very fine condition. Sweetened condensed whey kept well in sealed containers at room temperature for several months. It could be whipped to 200 or 300 per cent overrun. Drainage did not occur before eight or ten hours. The taste of sweetened condensed whey itself was too salty to be pleasing but it acted as a satisfactory base in some new whey foods.

A new porous candy filling was produced by dehydrating whipped, sweet whey at 70°C.(158°F.). When this product was chocolate covered, it kept well in an open room for several months. Candy of this

type, consisting of half whey solids and half sugar and containing nuts and a little cereal to lower the salt taste, made a very nutritious and attractive confection. These "Wheyfers" contained:

Whipped sweet whey.....	80%
"Pabulum" or other cereal.....	5%
Chopped nuts.....	15%

Whipped sweet whey was found to have some possibilities when flavored and used as an icing, a topping for soda fountain dishes, or as a garnish in hot chocolate.

WHEY FRUIT MIXTURES

Extensive experimental work was undertaken in an effort to make attractive products with whey solids and fruits. In some of these the foaming property of whey was valuable.

When milk or cream was mixed with acid fruit, a coagulation of casein occurred, especially when an attempt was made to heat the mixture. Whey protein was not precipitated by acid fruit unless the temperature was raised above the heat coagulation point of milk albumin. Whey-fruit mixtures showed only slight coagulation when pasteurized at 60 to 64°C. (140 to 147.2°F.) for 30 minutes. When the mixtures were processed more severely, as was necessary for packaging in tins, a substantial agglomeration of protein occurred. Nevertheless, in the presence of fruit pulp the coagulum was scarcely noticeable.

Fruit drinks were made by mixing fresh sweet whey with fruit juices and processing the mixtures in tins. Cheese whey did not give to these products sufficient distinction to make them of great importance although it did contribute to the nutritive value of the beverages. Prune juice was prepared by cooking and extracting prunes in separated fresh whey in place of water. The process coagulated the whey protein but the pulp and protein were removed by filtration. The resulting juice remained fairly clear during processing, and an agreeable drink was obtained. The whey contributed lactose, milk salts, and non-protein nitrogenous compounds to the beverage besides improving its flavor.

Mixtures of fruit juices and whey were simple to prepare when processing was not used. Good, nutritious, low-cost drinks were obtained but it cannot yet be generally said that from a flavor consideration they were more attractive than the plain juice.

A new canned fruit whip was made using fruit, sugar, gelatin, and sweet whey or whey powder. The mixtures, adjusted to pH 4.5, were processed in tins and remained practically unchanged during storage

for three or four months. The whips were capable, because of the presence of both whey solids and gelatin, of swelling to over 100 per cent overrun after whipping for two minutes. The foams possessed sufficient stability to set to a permanent whip during cooling in a refrigerator immediately after whipping. If, after cooling, the whip was mixed with cold whipped cream, it could be frozen without stirring into a smooth ice cream. A gradual deterioration in whipping properties during storage in the can has not yet been overcome. However, canned fruit whips were satisfactory when the storage period was limited to four months.

The following is an approximate composition of canned fruit whips:

Fruit juice and pulp.....	25% to 50%
Whey solids.....	14%
Sugar.....	18%
Gelatin.....	1%

To use: Whip product two to three minutes, place in serving dishes, chill well in refrigerator, and serve.

Note: This is an approximate formula only. Adjustment of the fruit and sugar content is necessary with different fruits.

Fresh fruit whips could be made in any restaurant or by any housewife simply by the use of sweetened condensed whey or whey powder and gelatin in place of the conventional egg white.

A modification of these fruit whips was made by substituting tomato and vinegar for fruit and sugar. The resulting products were whipped, poured into suitable molds, cooled, and used to serve with lettuce as a whipped tomato salad.

Sweetened whey or whey powder mixed with jam was found to produce preserves which could be whipped to at least 100 per cent overrun. The whip was stable for about one week. The use of six per cent whey solids was sufficient to give good whipping properties to the jam without having sandiness develop. Whipped jam is suitable to garnish soda fountain dishes, for waffles, or for desserts where a sweet sauce is required. Because of its high air content it lacks the intense sweetness and richness of ordinary jam.

Attempts were made to can fruit-flavored whipping cream and home ice-cream mixes. Casein-free whey cream was used as a source of fat. While these products were excellent when fresh, after a few months' storage a heavy fat separation occurred. A tendency for the fat to become rancid slowly was noted. These defects have not yet been overcome.

Attempts are being continued in an effort to introduce whey into other foods where its presence would seem to be advantageous.

The following are abstracts or abridgments of papers presented at the conference :

Packaging Materials and Their Application to the Food Industry.

ALLEN ABRAMS, Marathon Paper Company, Rothschild, Wisconsin.

Food manufacturers are constantly improving the cleanliness, flavor, and nutritive value of their products. The manufacturer of packaging materials is called on to help deliver food products to the consumer in prime condition, to prevent loss of flavor, penetration of molds or pests, and deterioration by light, air, or moisture change.

In the search for improved packaging materials a new composition, Paraweld, has made possible some interesting developments. This composition is tasteless and odorless, flexible, thermoplastic, and waterproof. It may be formed into a film or used for laminating or coating.

When formed into a self-sustaining film, the product is termed Parafilm. Such a sheet is highly flexible and stretches readily but does not return to its original length. It has remarkable resistance to water and moisture. With the aid of Parafilm, natural cheese is now being put up in an attractive, identified package, fully protected from loss of moisture or growth of mold. This development should bring about revolutionary changes in the manufacture and merchandising of natural cheese.

The Paraweld composition, when heated, may also be used for joining together papers, transparent films, and other types of sheets. The resultant sheets are highly moisture-proof yet may be scored, printed, glued, and otherwise converted like regular papers. Consequently these sheets have found immediate use for moisture-proof envelopes and bags. They have been employed as protective wrappers on such diverse products as gelatin powders and cake soap.

When the Paraweld composition is coated on paper or other sheet material it produces a flexible, waterproof, and moisture-proof coating. A unique feature of these sheets is that they may be heat-sealed readily to themselves or to other surfaces. They may then be converted into heat-sealing envelopes or bags suitable for packaging either wet products or those in which the original moisture content is to be retained. Examples of these packages are drink powders and powdered coffee. The sheets may be sealed directly against food-stuffs which are to be kept free of mold or moisture loss.

Food Preservation in Modern Glass Containers. H. A. BARNBY, Owens-Illinois Glass Company, Toledo, Ohio

The established principles underlying food preservation are generally understood. These are basic and, as such, relate more to the product in question than to any particular type of container. This means that any choice between preservative methods, such as heat sterilization, salt brine, acid fermentation, dehydration, etc., would be principally governed by the article of food rather than what it is to be packed in.

When we set about applying these well-established processes to commercial food preservation, however, we find that the container characteristics have a very definite bearing on the several operations and their ultimate success. One invariably meets the stern commercial realities of cost, speed, production efficiency, mechanical strength, positive seal, corrosion resistance, product appearance, etc. In other words, the physical, economic, and performance characteristics of a container are so vital in determining its ultimate acceptance for food packing that the container manufacturer's assignments are thus well defined. Besides knowing first hand the products and processes of the customer, his research and development efforts must seek to better existing container performance and economy.

GLASS CONTAINERS NO EXCEPTION

In recent years the glass container has come in for its share of improvement. It is the sum total of those advances relating to a more successful glass package which justifies the designation of "modern glass containers." Let us look at some of the milestones of recent years that mark such improvements.

One of major character is that of weight reduction with its attendant advantage of freight saving. If we take as representative the Stubby and Steinie beer bottles, we have a good example. These new shapes represent a weight saving of about 26 to 29 per cent and a height reduction of 30 per cent over the original shape. Significant to note here is the fact that this marked weight reduction has not jeopardized the physical strength of the bottle. On a check-up made by a beverage cap manufacturer, the Stubby bottle showed a vertical crushing strength of 3,500 pounds minimum to 7,000 pounds maximum.

About a year ago requests were received from certain brewers for a one-half gallon capacity bottle for marketing pasteurized beer. Through proper coördination of glass-forming technique and contour studies in relation to strength, the desired bottle was produced

and is now being successfully marketed. Anyone not familiar with brewery practice should stop and reflect on the rigors of temperature, pressure, and impact that such a bottle must undergo not once but many times during its life.

More recently through studies made on equipment and handling methods, we have succeeded in passing 20-ounce food jars through a continuous automatic reel-type sterilizer and cooler at a speed of 130 units per minute without any increase in breakage over normal. Actual breakage figures taken during commercial runs in this equipment have been strikingly low, only .04 per cent or four jars in 10,000 units packed.

Some of you may have visited certain tomato-products plants recently and witnessed the filling and capping of 220 bottles of catsup per minute. Bear in mind that these bottles, though preheated, are filled at boiling temperature and capped under a 400-pound impact pressure from the sealing machine. It wasn't so long ago that 90 bottles per minute was top speed for such a container. Certainly one of the bottles as made in the 90-per-minute day would not do the present job with any degree of satisfaction.

WHAT IS BACK OF SUCH IMPROVEMENTS?

Advancements in container performance, such as noted, were not due to chance. They are the result of a systematic study in three directions: properties and forming behavior of glass, contour in relation to strength, and improvements in certain container handling equipment. In the interest of conserving time we will refer only to the *first* of these.

One is apt from sheer habit to think of glass as one of the most brittle substances if he has not actually handled some of its more recent forms. Take, for example, glass thread or fiber from which a rapidly increasing variety of commercial products is being made. In one form the appearance and feel of this thread is identical with silk, even to the property of pliability. A single fiber of this thread is so fine (approximately .0002 inch) that it would require a length from London to Shanghai via New York to weigh one pound. The continuity of fibers in this yarn is apparent from our having produced commercially one strand 400 miles long without a break.

Some of the useful applications of fiber glass in various sizes are as filter cloth for mineral acids, etc.; electrical insulation (wire covering); battery separators; air filters; cellular acoustic ceiling blocks; and all types of insulation.

Unless one has actually seen these various products in use, it is hard to appreciate what utterly new properties not commonly associ-

ated with glass this fiber material has, such as flexibility, softness, and elasticity. In fact, the only reason for making reference to these new forms of glass has been to offer tangible evidence that glass as a material possesses marked resilience and flexibility. From the theoretical and applied studies leading to their development, even those persons most familiar with glass have had to change their thinking about the substance.

It may seem a far cry from glass yarn to the preservation of food in modern glass containers. The connection, however, is not difficult to trace. In the high-speed commercial preservation of foods the glass container is subjected to ever-increasing quality and strength requirements. Only glass containers that are properly made can satisfactorily meet these standards. The fundamental study of glass made necessary in the successful development of these many new forms has and will continue to be reflected in better containers for the food-packing industry.

Present Trends in Canning. A. W. BITTING, San Francisco, California.

It is rapidly becoming a dictum that canned foods must be prepared and preserved in a manner to hold their original natural qualities as nearly as possible, that is, with respect to composition, flavor, and vitamins. It is further recognized that while heat, the essential factor in sterilization, produces some changes in these qualities, they are in line with those to which we are accustomed in the usual preparation of fresh foods for the table. Efforts are now being made to effect less change in canning than in ordinary cooking. This is a very different attitude from that held at the beginning of this century when nearly all effort was directed to the one end to make foods keep, or to the attitude even 20 years ago when some advocated that all fruits and vegetables slightly alkaline or even of low acidity be subjected to a temperature, when cooking, sufficient to destroy the most heat-resistant strain of a certain microorganism irrespective of the effect upon the food itself.

The new viewpoint is more in accord and harmony with scientific cookery which strives to retain the maximum of body-building qualities and also those qualities which gave canning such marked advantages over drying, salting, preserving with sugar, pickling with vinegar, and smoking—processes which greatly altered composition and flavor. Appert, having been a chef, had an advanced viewpoint and sought through every means open to him to retain the highest qualities of the fresh material, though most of his followers for 100 years were content to spend their best efforts merely to insure keeping, and when this was attained they apparently felt that the end had been

accomplished. We are now beginning to look upon that as an essential but not the final step.

The present trend toward conserving natural qualities is progressing at a rapid pace. It can probably be best illustrated by citing two examples, the canning of milk and fruit juices. Both are liquid with solids in suspension but they require widely different cooking requirements for their preservation.

Milk is difficult to can because of the entrance of heat-resistant organisms on the one hand and its sensitivity to outside factors on the other. It was not until after 30 years of trials with varying time and temperature, and finally the addition of agitation during sterilization, that evaporated milk could be conserved in cans in a form to be acceptable in cooking and for use in coffee and tea. It has taken an additional 40 years to reach the present state of perfection, and strangely enough this has been attained primarily in the endeavor to cut costs in sterilization by converting a batch method into a continuous operation. It was found that introduction of cans of evaporated milk directly into a pressure cooker yielded a product inferior to the batch method; this in turn led to an investigation upon rapid, evenly conducted heating of the milk, holding it for varying short periods in a forecooker before delivering it into the retort, continuing the heating in the latter only long enough to sterilize, and subsequently cooling it very quickly. The results were so striking that they led to extended work on the effect of the temperature used in the hot-well prior to evaporation. Some laboratory work along this line had already been done but not applied practically. Now rigid heat control is followed in every step from the hot-well through the evaporator, the forecooker, and the sterilizer, with results that are constant and better than those previously obtained. Irradiation has been added, and now canned milk is suitable for almost every culinary purpose. It is sterile, has a bright color, uniform consistence, no separation of fat, and the change in flavor is reduced to nearly that of pasteurized milk. Within a few years practically 80 per cent of the output has been changed to this method of preparation. The initial object, that of lowering costs, has become of secondary interest to better quality. Of special significance, however, is the fact that we now have a flexible method of heat control from very low pasteurization through any sterilizing range that may be desired, and research indicates that improvements are expected in other lines.

Grapefruit juice is fairly typical of the fruit-juice group and contains some fine fiber suspended in a clear liquid. When obtained from sound, fully matured fruit, the juice is low in organisms and, owing to the high natural acidity, they are easily destroyed. The problem

then reduces itself to handling the fruit so as to exclude extraneous organisms, extracting the juice without sufficient pressure to release the bitter principle from the white connective tissues surrounding the segments and inner lining of the peel, vacuumizing at once to get rid of air and other gases or conducting the work so as to avoid action of oxygen upon the juice, filling into cans in the shortest interval, cooking and cooling promptly. The flavor, which is the most important quality to conserve, is easily affected by heat, which if carried too high or continued too long induces bitterness and astringency. The work must therefore be conducted just below the border line, which fortunately in this instance is above that necessary for sterilization. The work with other juices follows the same principles though varying considerably in details.

What has been learned is that delicate juices from fully matured fruit can be handled so as to retain a large proportion of their natural qualities and thus there is created a demand for them on the part of the public, whereas only a few years ago they were not marketable. The four important juices have been built up to a production in excess of twenty million cases in a remarkably short time and the range is being extended to include apricot, peach, pear, plum, and other juices. A more diverse range has been packed this year than at any previous time. The importance of this development lies in the fact that the products are non-competitive with the usual canned fruits and in addition point a way in which the latter may be improved.

The successful preparation of these juices is only partly due to the improvement in technique; it extends to the fundamentals of selecting well-ripened, sound stock of the proper varieties to obtain the best flavor. Some varieties of oranges are a failure for juice purposes, a fact which is in line with results obtained upon other fruits used in canning. In most cases the amount of heat necessary for sterilization causes objectionable changes in the flavor of oranges. The same thing is true for some varieties of peaches, though in others the heating may have the opposite effect. The off-flavor may be slight at first but become more objectionable with time. At present there is an unusual amount of activity going on among chefs and horticulturists in the selection of strains of fruits and vegetables which respond most favorably to this requisite preparatory treatment. The rule to use more mature fruit is being tried out cautiously in canning. The hard, ripe fruit permits sharp knife-edges on the pieces, a lighter and often a more translucent color to attract the eye, and a clear sirup but, lacking in the expected fine flavor, does not make a strong appeal to the taste. The canner operates on the basis that hard, ripe fruit is more easily

handled and that the heating will soften the tissues, but the method does not supply an undeveloped flavor.

The trends toward retention of natural qualities are not limited to selection of raw materials and technique of operations but are being extended to the kind of apparatus used in the preparation so that no objectionable reaction takes place between the apparatus and the product being treated. The great copper kettles and vacuum pans are giving way to nickel, stainless steel, glass-lined tanks, and other materials resistant to chemical action. Galvanized iron in mixing, sirup, and brine tanks is no longer approved and is even being eliminated from blanchers, steamers, and other apparatus where it may come in contact with the products being treated. Very small traces of metal may cause appreciable changes in products though they cannot be easily measured by methods now available.

The perfect container has not arrived but is well advanced on the way, for the glass jars and metal cans of 25 years ago would not be accepted at present. Large sums have been expended for research upon both with excellent results. Glass jars are being manufactured with greater accuracy as to measurement, uniform thickness, weight, resistance to shock in handling, and temperature changes. The containers are better designed to work through the machines and conserve space in storage and shipping. The base metal in the tin can has been altered to be more resistant to organic acids so that corrosion and "pinholing" are now minor problems. Metallic and astringent flavors are no longer tolerated without protest, which means that new lacquers must be and are being devised to resist special products and not merely to act as inside coating. The asphalt-lined can patented almost 60 years ago to hold shrimp was a very crude forerunner of the specially coated containers now used for various products, and which make possible the canning of new items, such as orange juice, pineapple juice, and beer.

The trend of the times is for better foods in greater variety, selected both for a balanced sustenance and the pleasure which they afford at the table. The trend in canning is to conserve perishable foods or to prepare them for use in a higher degree of perfection and safety to meet the exactions of the consumer, dietitian, and chef.

Current Technological Problems in the Dairy and Ice Cream Industries. A. C. FAY, H. P. Hood & Sons, Inc., Boston, Massachusetts.

MILK PRODUCTION

Use of Lactogenic Hormones: Of particular interest in the field of milk production is the recent use of pituitary hormone for stimu-

lating increased production of milk in animals from 10 to 350 per cent. Although the composition of the milk produced is normal, the effect of the lactogenic hormone is most marked in animals that are well-fed for high-production levels.

Mastitis: The more one learns about mastitis the more pessimistic he becomes as to the practicality of wholesale elimination of the disease from all dairy herds. The extraordinarily high percentage incidence of the disease coupled with its insidious dormancy in apparently normal animals renders the control comparable in its complexity to that of eliminating the common cold in humans.

Fortunately, only a decimal percentage of existing mastitis is caused by organisms pathogenic for humans. This fact, however, should not be construed to imply that septic sore throat traceable to milk from certain cows is a rare disease. Statistics from many states rank this disease high in the list of infectious and controllable diseases. Although pasteurization is the most practicable means of preventing the spread of septic sore throat through milk, it is still highly desirable to eliminate cows with infected udders for economic, esthetic, and public-health reasons. It is safe to predict that dairy-control laboratories in the future will be devoting an increasingly greater proportion of their effort to the study, control, and elimination of mastitis.

MILK PROCUREMENT

Resazurin Test: Considerable interest is now shown in the use of resazurin as an oxidation-reduction indicator which is sensitive at higher levels of potential than methylene blue. Results indicate the possibility of detecting raw milk with a bacterial population in excess of 200,000 per milliliter within one hour. Since this dye reduces at levels of potential only slightly below the normal oxidation-reduction potential of milk, and is sensitive to slight decreases in that potential, further work needs to be done to establish three fundamental points: (1) the extent to which there is uniformity of variation in the oxidation-reduction levels of various samples of milk, (2) the extent to which there is uniformity or variation in the poisoning properties of milk, and (3) the variation in the reducing intensities of different organisms normally present in milk.

PROCESSING

Pasteurization Test: The ideal test for pasteurization would necessarily be dependent upon a normal constituent of milk which would be present in uniform amounts in all milk and whose resistance to heat is just at the threshold of the thermal exposure employed for pasteurization. The nearest approach to satisfying this ideal and this need seems to be the phosphatase test which is receiving considerable

attention in dairy-control laboratories. Milk which has not been properly pasteurized or milk to which only a small amount (.1 per cent) of raw milk has been added subsequent to pasteurization contains detectable amounts of the active enzyme phosphatase. On the assumption that the rate of inactivation of the enzyme would be uniform at the fixed thermal exposure of pasteurization, the amount of enzyme present at the end of exposure would obviously depend upon the amount present in the raw milk. Recent work has shown that mastitis induces abnormalities in the phosphatase content of milk. Further work needs to be done to establish more convincingly that the quantity of the enzyme in different samples of raw milk is sufficiently constant to avoid misinterpretation of the results of the test.

Pasteurization of Ice Cream Mix: Experimental work carried on coöperatively at the Kansas and Iowa Agricultural Experiment Stations has shown that the sugar present in ice cream mix tends to protect organisms against the destructive action of heat. If heat induces the physical process of coagulation of cell protoplasm, it is logical to expect the rate and hence the degree of coagulation to be altered by substances such as sugar which exert osmotic effects at the cell surface.

According to this concept, the presence of sugar in ice cream mix tends to retard the rate and prevent the degree of coagulation of the cell from advancing quite so far in the direction of the irreversible stages. This point of view also affords an explanation for the frequent observation that bacterial counts of ice cream are considerably higher and more uniform when plated on a medium containing some carbohydrate than when plated on plain agar. The carbohydrate medium is more capable of peptizing the partially coagulated cells protected by the sugar in the mix than is the plain agar.

These experiments suggest caution to the dairy-control laboratory in the selection of media for the plating of ice cream and in making thermal-resistance studies of organisms suspended in such dairy products as ice cream or sweetened condensed milk.

Homogenization of Ice Cream: Studies of the physical chemistry of homogenization and its effect on the dispersion of fat as well as the surface adsorption phenomena have been greatly facilitated by the use of photomicrographs. Commercial use of photomicrographs of mixes homogenized under different conditions gives promise of more intelligent control in the direction of standardization of products made from day to day or manufactured in different plants but sold under one trade name.

BACTERIAL CONTROL

Colon Counts: The determination of the numbers of colon-aerogenes bacteria in dairy products is receiving more attention each year.

Although the acceptance of colon counts as an index to poorly pasteurized milk has met with considerable objection, it is now rather generally conceded that the presence of colon-aerogenes types in pasteurized dairy products justifies immediate investigation. Contamination from poorly washed equipment subsequent to pasteurization is frequently detected by means of colon counts. They are of particular value in the detection of sources of contamination which contribute relatively small numbers of bacteria. Owing to the low order of magnitude at which colon counts are interpreted, the addition of a relatively few colon types by a piece of equipment may increase the colon count several fold although the actual increase in the total bacterial count may not be detectable.

The high temperatures now commonly employed in the pasteurization of ice cream mix and of cream for bottling practically preclude the survival of colon-aerogenes types in these products. The presence of these organisms in the finished products which have been pasteurized at such high temperatures is almost certain evidence of contamination subsequent to heating. Dairy-control laboratories are finding, and will continue to find, increasing use for colon counts in the analysis of dairy products.

Use of Tryptone-Glucose-Skim Milk Agar: Perhaps the greatest interest of dairy-control laboratories is now centered around the proposed adoption of tryptone-glucose-skim milk agar as a substitute for the present standard beef-extract agar now recognized by the Committee on Standard Methods for milk analysis of the American Public Health Association. It is also proposed that plates be incubated 48 hours at 32°C. (89.6°F.) instead of 37°C. (98.6°F.). The new medium and method of incubation permit the growth of many organisms incapable of development on plates made from the present standard medium and incubated at 37°C. This is particularly true in the case of milk with high bacterial counts and also with ice cream in which the sugar has exerted a protective effect on the bacterial flora during pasteurization.

Development of a Fiber Container for Fluid Milk. F. F. FITZGERALD, American Can Company, New York City.

In many cities and localities the law requires that milk be sold in retail containers. This requirement has been made from a public-health standpoint, because authorities have recognized the relation of the milk industry to public health.

Since the largest proportion of the business of can manufacturers in the United States is the development and sale of individual single-trip containers for the factory packaging of food products, and since

raw milk represented a possible market for such containers, the American Can Company believed it advisable to investigate the volume and character of milk production and distribution.

Such an investigation, conducted by the Marketing Division of the American Can Company, indicated 12 billion quarts as the yearly current volume of fluid milk consumed in the United States as a whole. Of this total, 8 billion quarts are sold in urban and suburban centers. The urban market of 8 billion quarts is mainly concentrated in the 96 metropolitan districts of 100,000 or more population, which consume 77 per cent of this amount, leaving 23 per cent to the smaller suburban centers.

There is little seasonal trend in fluid milk sales. The U. S. Department of Agriculture figures on fluid milk delivery in New York, Boston, and Philadelphia, show a minimum of 8.05 per cent of the total yearly sales for December and a maximum of 8.71 per cent for July. New York City alone consumed 1,392,657,000 quarts during the year 1930, of which 905,227,000 were delivered and 487,430,000 were obtained through stores. The rapidly increasing proportion of milk sold by stores was especially interesting from the standpoint of the container manufacturing industry.

With these facts in mind it was decided to investigate the styles and shapes of milk containers being used with the object of buying the rights to manufacture any package of suitable design then on the market. The survey did not disclose a package that answered the requirements and it was decided that experimental work should be conducted to develop such a container. This work was carried on for practically five years.

Single-trip paper containers for milk appeared on the American market in the early 1920's. Although the use of such containers is, therefore, not new, their development today is a subject of serious study from both an economic and a health standpoint. Such a study has been, in fact, long overdue. Paper wrappers, paper dishes, and all sorts of containers have been in wide use for foods, with comparatively little attention given to the sanitary conditions under which the paper and container are manufactured and to the bacterial condition of the container with special reference to proper food handling and preservation.

At present most types of retail containers are selected or designed for a specific product. A particular design or form may be chosen for different reasons, such as sales appeal, economy in price, advertising advantages, packaging and shipping characteristics, and adaptability to preserve the product properly. The fiber milk container finally adopted by us was similarly designed to permit the economical

packaging, sale, and delivery of milk from the dairy to the consumer in the most sanitary and satisfactory manner.

We chose a single-trip container, first, because such containers require no deposits at stores and they do not have to be saved by the consumer. On the contrary, they are destroyed by the consumer and the dairy is thereby kept bacteriologically free from contact with the home. Any connection between the home and dairy is a special public-health hazard if there are cases of communicable diseases in the home. Second, a single-trip container obviates the recleaning and sterilization that are necessary with re-used containers.

We chose a fiber container because of its low cost, saving in weight, and its easy destructibility after use, and because the use of a non-transparent container protects the product against deterioration owing to sunlight.

The package was so designed that it could be made with minimum mandrel forming, which usually retards speed of production. A square design was selected because such packages could be packed solidly in a shipping carton, thus economizing space and offering insulation against rise in temperature during the transportation of filled containers.

We adopted a particular form of closure in which the closure cap is integral with the top and cannot be entirely removed from the package. This form has various advantages. The cap may be closed under sterile conditions in our factory to prevent contamination of the container from the time it is made in the factory until it is used in the dairy. The cap may be mechanically opened in the dairy and the container resealed after filling. As the cap is integral with the top, it cannot be removed entirely from the package and is always available for reclosing in the home. The container possesses a no-drip feature, i.e., the milk does not run down the sides of the container after the desired quantity has been removed.

The character of materials used in the manufacture of these containers, as well as the manufacturing methods, contribute to making them satisfactory and sanitary containers. Only paper made from virgin chemical or mechanical pulp is used, and methods of wrapping and handling such paper stock with the minimum of manual contact were devised to prevent contamination by human touch. The adhesives are thermoplastic cements, the use of which avoids other types of adhesives that might themselves be a medium for bacteria unless preservatives are employed.

The containers, after being formed, are treated with paraffin with the object of making the package stronger and more rigid, improving the appearance, providing waterproofing, providing hermetic seal, and assisting in sterilization. Paraffin treatment was adopted because it

attains these objectives; paraffin can be made free from odor and taste and it is inert to bacterial attack.

The individual fiber containers are delivered to the dairies in sealed, dust-proof paper cartons. This assures the delivery of clean containers to the dairy and provides protection to containers held in storage before use.

Much interesting work has been done on the bacteriological aspect of milk containers. Originally the work was largely carried on in our laboratory. Later we had the valued coöperation of Dr. F. W. Tanner of the University of Illinois, and later we were privileged, together with others, to coöperate with Dr. R. S. Breed of the Geneva (N. Y.) Agricultural Experiment Station.

During the early days it was believed that the use of a paraffin bath operated at about 82.2°C. (180°F.) would by itself guarantee a sterile product. This has been disproved. There is a marked reduction in the number of viable organisms if they were originally present, but a small percentage may survive. The paraffin does, however, play a role in keeping many bacteria from getting into the milk, and may be said to inactivate the majority. Interesting data have also been obtained as to the gradual death of bacteria in the unfilled, paraffined containers, probably because of unfavorable growth conditions.

The bacteriological condition of the fiber milk container has been carefully followed both during its development and especially after it was put into practical use in New York City. These studies carried out by Drs. F. W. Tanner, Evan Wheaton, and F. M. Clark have now been in progress for some four years, during which time thousands of containers have been examined. Modifications of the American Public Health Association rinse test, as published in the last issue of *Standard Methods of Milk Analysis*, were used. A report of the relative merits of several methods will be published later. It is believed, however, that a rinse test is the best method for following the bacteriological conditions of the fiber milk containers.

Data from these examinations allow clear-cut conclusions. Fiber milk containers are now being supplied to dairies in a practically sterile condition as determined by a rinse-test method approved by several laboratories. Eighty per cent of fiber milk containers now being used yield no viable bacteria. Of the remaining 20 per cent that do show colonies on plates made with rinse water, over 90 per cent give fewer than five colonies per bottle, the great majority showing but one colony per bottle. These counts are especially significant when it is realized that the Public Health Ordinances and Codes (1935) state that bottles shall be so clean that they shall contribute not more

than approximately 1,000 organisms to each quart of milk, or one bacterium or colony per cubic centimeter of capacity.

The types of bacteria have been carefully observed. *Escherichia coli* has been consistently absent when determined by culturing five c.c. of rinse water in lactose broth. Lactose fermenting organisms have never been found to be present. Bacterial types that have been found on the plates have been white staphylococci, yellow sarcina, and spore-forming rods. Rarely have molds been isolated. Consideration of the types of organisms that have been isolated, along with the fact that they are present in very small numbers in containers that have any viable microorganisms whatsoever, indicates that real progress has been made toward an ideal package for fluid milk. Further study should even improve the present situation.

Dr. R. S. Breed and Dr. J. R. Sanborn, of Geneva, have pioneered in developing the principles of sanitation that should be observed in the manufacture and use of fiber containers for milk. At a conference held in July at Geneva with paper manufacturers, container manufacturers, and health authorities present, the suggestion was made that containers should be of virgin chemical or mechanical pulp, and that a provisional tolerance of colonies of microorganisms per gram of disintegrated board be set in order to establish sanitary conditions of paper manufacture. The paper, whether delivered in sheet form or in roll form, should be properly protected by wrapping to avoid contamination in shipment from the paper mill to the converting plant. Likewise, the paper should be handled mechanically as far as possible in the converting plant to avoid manual contamination.

Regulations were suggested to cover the efficient moisture-proofing of the containers and the use of sanitary adhesives. It was recommended that the use of germicides in manufacturing the containers be prohibited. Suggestions were made as to the proper handling of shipped filled containers.

They finally suggested the same standards for examination of the fiber container as for bottles, as now determined by the standard methods of the Public Health Association, although they stated that sanitary conditions in pulp and paper mills, container-manufacturing plants, and dairies usually enable paper containers to meet a very much more rigid standard.

The adoption of the fiber milk container should in each instance be preceded by a careful survey of the methods of packaging, sale, and distribution of the product. These methods must be adjusted, if necessary, to capitalize all the inherent advantages of the package to make its use an economic success.

The suitability of our present container is evidenced by the fact that we are now supplying millions of containers weekly to the dairies of the New York Metropolitan district. This quantity at the present time is the limit of our productive capacity but still fails to supply the demand of the trade.

Research Problems of the Can Manufacturer. WILLIAM H. HARRISON,
Continental Can Company, Chicago, Illinois.

In the can-manufacturing industry it is the usual custom to classify containers into two groups—Packers' cans and General Line cans.

Packers' cans are the hermetically sealed cans, mostly cylindrical in form, customarily used for the canning of fruits, vegetables, meats, fish, and milk. Numerous other items are also canned or packaged in these containers.

Under the classification of General Line cans are those of various styles, sizes, and shapes used for the packaging of both dry and liquid commodities, the number of which is infinite. Included are the cans used for packaging of such preserved or otherwise prepared foods as candies, nuts, and nut butters; coffee, cocoa, tea, and spices; honey, molasses, and syrups; lard, salad dressings, and shortenings; biscuits, cakes, crackers, and doughnuts; frozen fruits, frozen vegetables, frozen eggs, and frozen cream; fats and oils; beer and the cheese and pretzels to eat with it, ales, and other beverages.

Primarily, the function of a can company is to manufacture cans and see that they are delivered to its customers and to supply the desired equipment for sealing the cans in the plants in which they are closed. The can maker is neither a processor nor a distributor of the commodities packaged in the containers which he supplies. He sells no commodity to the consuming public; nor is the consumer interested in the container so long as it delivers the contents in the most satisfactory manner, is bright and attractive in appearance both internally and externally, and has no bad effect upon the contents. Being in this position, a can company is dependent, in a large measure, upon the users of its cans to increase can consumption. Aiding this consumption is a secondary function of a can company. It is mutually profitable.

The principal channels through which a can manufacturer may obtain additional can business are the following: finding new uses for established containers, developing new containers for products for which established containers are not suitable, and increasing consumption of commodities packaged in established containers.

In brief, it is for the purpose of handling the technological phases of these activities and of can making that the can companies have

established their research departments. Differences in the character of the work are such that it has been found desirable to classify it into two groups: (1) those matters related to sales, or utilization of containers, and (2) those related to can-manufacturing operations. Under each of these major divisions there are several sections into which are classified, and to which are assigned, the activities that are alike or similar in character.

It is in the work related to increasing consumption of commodities packaged in established containers that the research department of a can company finds one of its most fruitful fields. Since the canning industry presents a very broad one, and in it the research facilities can be employed to their fullest extent, reference to some activities in this field will be used to illustrate the application and results of the work.

The problems arising in the canning industry, in the solution of which the research departments of the can companies coöperate with the canners, may be classified into three groups: (1) those which affect the industry as a whole, either directly or indirectly; (2) those which affect but one item or a class of canned foods; and (3) those which relate specifically to an individual canner.

Since success in canning is so dependent upon the character of the available farm and orchard products, the research departments of the can manufacturers utilize the services of experts in agriculture. The chief purpose for employing these specialists is to promote improvement in the quality of the raw products which are canned and to aid in the abundant production of them economically. This is done by maintaining contacts with the various experiment stations of the United States Department of Agriculture, the agricultural colleges engaged in research on fruit and vegetable culture, plant breeders, and other similar agencies. Trial plots of seed companies and canners are visited, and the merits of new varieties and strains investigated. The southern tomato-plant-growing areas, from which plants are shipped to canners in the northern tomato-producing districts, are visited during the growing season and the fields and shipping facilities inspected. By these methods the required data are obtained promptly and are quickly made available to the industry. The secondary duties of the raw-products specialists include making service calls on customers having an agricultural problem calling for prompt solution, exploring new production territories for contemplated canneries, developing and disseminating information on effective methods of controlling plant diseases, and combating insect infestation.

The research departments of the can manufacturers coöperate actively with the research laboratories of the National Canners Associa-

tion in the solution of problems affecting the canning industry as a whole, those affecting a section or group, and those affecting the canners of a specific commodity.

National Canners Association Bulletin No. 26L, available to all canners, contains recommended processes for the principal nonacid canned foods. They are based upon a mass of heat-penetration data, information on the results of resistance of spoilage organisms, results of studies of commercial practices, etc., accumulated by the research departments of the can manufacturers during the course of their research and service activities, as well as on data similarly obtained by the Association. The publication also contains recommendations on equipment for processing retorts and other recommendations similarly based.

Obviously, spoilage of commercial significance in canned foods has an adverse effect on the industry as a whole. A great deal of work is done by the research departments of the can manufacturers which is effective in minimizing such spoilage.

The McNary-Mapes standards of the items for which such standards have been promulgated have resulted from much scientific work to which the research departments of the can companies have contributed no little part.

Many other food industries are using methods employed by canners for controlling cleanliness in the manufacture of tomato and other similar products. The staff microscopists of the research departments have rendered material assistance to the industry by instructing cannery technicians in mold count, insect fragment count, and similar determinations. Frequently schools of instruction are held on subjects relating to canning operations. These are held in coöperation with canners associations and the State Agricultural Colleges.

Studies are made on sources of contamination resulting in poor or off-flavors, undesirable color, spoilage, etc. Usually these studies are made for the purpose of eliminating the undesirable results being obtained in a specific cannery. The results of such work are not published, but in time become available to the industry as a whole.

From a product which might now be described as "whole-grain corn of poor workmanship," there has been developed the Maine or cream-style corn and the whole-grain corn packed with free liquid containing sugar and salt. More recently a method for canning of corn whereby the can is sealed under vacuum was developed by scientific research. The new product is packed with only sufficient free liquid to be assured of adequate saturated steam in the can during the retorting process. Corn on the cob and whole-grain corn are

packed by this process. In this style of pack the diffusion of the flavor from the kernels into the liquid is reduced to a minimum.

Research on the effect of agitation during the sterilization of No. 10 cans of cream-style corn developed a product possessing the fine flavor and the fine color of this product in No. 1 cans.

The development of the canning of tomato juice has been the result of coöperative work done by the research departments of the can manufacturers with the canners of tomato products. This has resulted in a product practically unknown to the consuming public in 1927, becoming so popular that 11,500,000 cases were produced in 1936.

Large quantities of the juices of grapefruit, pineapple, oranges, and prunes and lesser quantities of the juices of lemons, limes, tangerines, the berries, cherries, etc., and the juices of some vegetables are articles of commerce. Research work by the can manufacturer has contributed no small part in the successful production of these commodities, and will play an important role in perfecting them in accordance with the apparent trade demand.

Considerable time is spent by chemists, bacteriologists, and microanalysts in developing new and in applying recognized analytical methods to the problems of food research. In such work unusual accuracy is frequently necessary. A vast amount of work is done on such analytical methods in coöperation with other research laboratories, and coöperative work is done with control laboratories on control methods.

Control over the quality of materials used in manufacturing cans is an exceedingly important function of the research department of a can manufacturer. A complete list of these materials with the specifications under which they are purchased would make a formidable book.

In 1915, coöperative research was started by the National Canners Association with the American Sheet and Tin Plate Company and the American Can Company, having for its purpose the improvement in quality of the tin plate being used in the manufacture of food containers. This was the beginning of intensive research which led to the production of "Type L plate," a cold reduced tin plate of very low metalloid content, highly resistant to the corrosive action of fruits and other acid foods. Cans made from this plate have very markedly lengthened the shelf life of the more acid canned foods. This development is one of the most valuable contributions which the manufacturers of tin plate and the can manufacturers have made to the public.

The main laboratories of research departments of the can companies are centrally located, but branch research and service lab-

oratories convenient to the fields of activity are maintained. One or more of the major can companies have laboratories at Chicago, Seattle, Portland, Oakland, San Francisco, Los Angeles, Houston, New Orleans, Baltimore, Syracuse, and New York City in the United States and at Hamilton and Toronto in Canada. In their size, the adequacy of their equipment and other research facilities, the number of trained scientific workers employed, and in the scope, quality, and quantity of work performed, the research departments of the larger can manufacturers rank among the foremost institutions of the country engaged in research in the problems of food preservation.

Engineering of Pasteurization. C. A. HOLMQUIST AND W. D. TIEDERMAN, New York State Department of Health, Albany, New York.

Starting with a definition of pasteurization such as "subjecting every particle of milk to a temperature of 143°F. or more for not less than 30 minutes, or to a temperature of 160°F. or more for not less than 15 seconds, in approved pasteurizing equipment," the task before the engineer is to design and build practical equipment to accomplish this purpose. The first application of this principle was by artisans having little knowledge of engineering. The situation was so bad that after participating in one of the first engineering studies of pasteurizing equipment, Phelps said, "... the lack of sound engineering in a distinctly engineering field has been conspicuous and rather deplorably so."

There were two general faults: first and most important, faulty design and construction, leaving avenues for viable pathogenic bacteria to get through; and second, faulty design and construction causing scorching of portions of the milk, damaging creaming, encouraging development of thermophilic bacteria, and producing similar undesirable effects.

Comprehensive engineering and bacteriological tests of commercial pasteurizers at Endicott, New York, beginning in December 1921, served to awaken health officials as well as the industry to the need for better engineering in this field. Later, Whittaker in Minnesota, Putnam and Frank in Chicago, and the New York State Department of Health did considerable work on engineering tests of commercial pasteurizers, using thermocouples to determine temperatures of milk at strategic points throughout the process of pasteurization. Among the serious defects revealed by these experiments were (1) dead ends, (2) leaky outlet valves, (3) leaky inlet valves, (4) contamination of pasteurized milk with raw milk trapped in submerged inlets, (5) under-heated foam on the surface of pasteurized milk, (6) sluggish recording and indicating thermometers difficult to read, (7) record-

ing thermometers badly out of adjustment with no means provided for checking, (8) indicating thermometers on which the metal scale had slipped with no etched mark on the tube for use in resetting the scale, (9) leaks in heat exchangers, (10) short circuits in continuous-flow pasteurizers, (11) inaccurate temperature control and lack of safeguards against low temperatures. These defects resulted in defeating the effect of pasteurization.

The remedies for these defects which engineers have developed are (a) valveless outlets or outlet valves installed flush with the inner wall of the pasteurizer and so built that milk tending to leak past the valve will run to waste, (b) valveless inlets or inlet valves protected against leakage and with air relief to permit milk which had passed the valve to drain into the pasteurizer, (c) foamless pumps, foamless inlet lines, or automatic foam heaters, (d) recording and indicating thermometers with $\frac{1}{16}$ -inch scale division per degree, (e) indicating thermometers so installed as to be used for operation and for checking the recording thermometer, (f) indicating thermometers with marks etched in the glass at pasteurizing temperature to aid in detecting and resetting scales which have slipped, (g) safeguards against leakage of unpasteurized milk into pasteurized in enclosed heat exchangers and similar protection of surface-type, regenerative heat exchangers by the use of leak-protector grooves and the extension of the ends of tubes beyond the collecting troughs using diverting fins at the end of each tube, (h) the designing of continuous-flow holders upon actual holding time determined by color or similar test, (i) the development of automatically controlled precision heating for continuous-flow pasteurizers, and (j) the provision of automatic devices for flow diversion of under-heated milk and automatic low-temperature milk-pump stops.

Some of the earlier devices, particularly outlet valves designed to correct defective equipment, were far from being satisfactory. Defects have developed and have been eliminated. The end result has been equipment that overcame the faults without developing new ones. Notwithstanding this development it is disconcerting to find that the modern vat pasteurizer is not as yet equipped with devices for showing positively the holding time at pasteurizing temperature. Furthermore, recent use of the phosphatase test for pasteurization indicates that further improvement is necessary and may be expected.

Along with this have gone other important engineering developments. Metals have been tested and new alloys developed in the effort to secure better milk-contact surfaces. Most modern pasteurizers have milk-contact surfaces of glass fused on steel or of stainless steel. Pulleys, shafts, and belts have given way to concealed motors for driving agitators. Welded joints have taken the place of soldered joints. Sur-

faces that need cleaning have been made smoother and more easily accessible. Covers have been constructed so that anything falling or dripping on them will fall on the floor instead of into the milk vats. Methods of applying heat have been so improved as to provide low-temperature differentials and to avoid surfaces with hot spots that scorch some of the milk. Milk pumps have been redesigned to minimize foaming, to facilitate cleaning, and to prevent damage to creaming.

While safety is the primary consideration, it has been necessary to accomplish this without destroying flavor or any of the good properties of milk. Early crude attempts at commercial pasteurization, resulting in scorching or in producing an oxidized or metallic flavor in milk, turned consumers against pasteurization and led them to seek raw milk that tasted better. Equipment is now available that will pasteurize milk with a full factor of safety without damaging the flavor or materially diminishing any beneficial properties.

Pasteurization of cream and other milk products is of considerable public-health importance. Recent developments have not only shown dangers in the consumption of unpasteurized products but have resulted in more general use of pasteurized milk and cream in the preparation of these products.

Problems Yet to be Solved in the Dairy Industry. H. F. JUDKINS.
Sealtest, Inc., New York City, New York.

Existing problems in the dairy industry may be national, sectional, or individual-company in scope. They have to do with production, procurement, processing, and distribution. To appreciate the wide range of problems that exist, one must have a picture of the size and scope of the dairy industry. There are approximately twenty-six million dairy cows in the United States producing annually slightly over one hundred billion pounds of milk. Nearly two million men are engaged directly in the producing and handling of milk on a commercial scale. The national farm income for milk and milk products is in the vicinity of \$1,750,000,000 per year, or somewhat better than 25 per cent of the total farm income.

The average person eats more than one ton of food per year, of which dairy products constitute about 45 per cent. The six main branches of the dairy industry are processing and distribution of fluid milk and cream, the manufacture of evaporated milk, butter, cheese, ice cream, and dry milk.

One of the problems existing in the fluid-milk business having to do with the production of milk on the farm is the uneconomical, low producing cow. The average cow produces in the vicinity of 4,000

pounds of milk per year, which is less than one-half of the amount required for profitable production. Great strides have been made in improvement of the quality of milk, but much remains to be done, especially with the keepers of small herds. The problem of finding a method of buying milk from producers that is satisfactory to all concerned is also still a major one.

Vast improvements have been made in transportation of milk in insulated tank trucks and cars. Great improvements have occurred in the handling of milk in plants, particularly in the development of stainless steel equipment. There is still room for improvement in the development of machinery for specific processes and in the simplification of the pasteurizing process.

Increased wages, the increasing tendency on the part of consumers to buy milk from the store and to use evaporated milk, and the keen competition among distributors, mean less milk delivered per route. This is presenting the very acute problem of ability to deliver milk at a price that will not result in decreased consumption.

The problem of handling surplus milk frequently is a serious one, and there is difficulty in getting consumers to understand why they must pay at least twice as much for their quart of milk as the producer gets for all of the milk that he sells. Likewise, the producer cannot understand why he gets only one-half of what the consumer pays for his quart of milk. The answer in a nutshell, of course, is that the milk distributor does not get the consumer's quart price of milk for anything like all of the milk that he buys from the producer. The surplus milk made into butter and other products brings much less money.

Evaporated milk, which is whole milk condensed approximately two to one, has been constantly gaining in favor, especially for cooking purposes. From 1920 to 1936 sales of this product increased three-fold. The outstanding problem in the evaporated-milk business is to develop a process which will result in a milk of normal flavor and color.

Ice cream is truly our national dessert as well as being a very valuable food. Improvements in its manufacture and distribution have been rapid. There is still considerable variation in opinion as to the best type of ice cream, particularly with respect to its butterfat content and texture, that will result in maximum consumption. Since ice cream is sold largely through dealers, the manufacturer is confronted with the problem of a rapid turnover of ice cream and the sanitation of the premises where it is sold.

With the improvement in quality of American cheese and with the development of innumerable varieties and ways of using cheese,

the consumption in the United States is on the increase, being in the vicinity of five pounds per capita annually. However, the cheese manufacturer has a very real problem to develop a consumption as great as that in some of the European countries, Switzerland for example, where consumption is about 16 pounds per capita per year. Increased consumption in this country will result from research on improved quality, keeping properties, and the development of new varieties.

Problems in the butter industry have been ably discussed by Mr. M. E. Parker.

One of the miscellaneous problems of the dairy industry is the development and selection of proper cleaning compounds for dairy equipment. Variation in the composition of water supplies in different parts of the country makes this a difficult problem.

Much work has been done also on the disposal of dairy wastes from country plants where sewers are not available. No real satisfactory, inexpensive method has as yet been developed.

The personnel problem, common to all industries, is a very important matter in the dairy industry where so much depends on doing the same thing in exactly the same way each time and doing it neatly.

Technological Exploration of the Art of Buttermaking. M. E. PARKER, Chairman, Research Committee, American Butter Institute, Chicago, Illinois.

It may not be incorrect to state that probably the more nearly chemically pure or refined the food product, the more susceptible it is to technological processing. Conversely, the more complex and the more nearly natural its composition, the more dependent is quality control upon the artisan's skill, but it can be rendered more certain by the application of the technologist's knowledge.

While buttermaking is not as adaptable to technological principles as is the manufacture of some other food products, it is by no means immune from profitable scientific investigation in the establishment of fundamental facts. It has, in fact, profited from the technological exploration of its production practices.

Less than 50 years ago the development of the centrifugal separator, first successfully manufactured by DeLaval in 1886, provided the basis of the factory system of buttermaking in this country, since his machine had reached the stage where it could be used on the farm. The introduction of these hand separators on the farm was slow at first, but it gained considerable impetus in the course of a decade, particularly in the middle western states. The creamery-butter industry was thus born with the advent of the farm separator, and the

art was gradually revolutionized. Large centralized creameries which secured their cream almost entirely from milk separated on farms and gathered their supplies by a variety of procurement systems, evolved in the natural course of events. Today, of the more than 4,000,000 farms in the United States, it is conservatively estimated that at least 2,000,000 of them are engaged in the production of cream for buttermaking. There are approximately 4,500 creameries churning butter, most of which are located in the Middle West, where the distance of the producing farmer from his market varies from a few to many miles—four miles in Minnesota to 40 to 50 miles in Texas—and the number of contributing farms from a few to thousands.

It is evident from these historical and geographical facts on the development of the creamery-butter industry that buttermaking during the past few decades has been developed by artisans. Even today there are striking differences of opinion among buttermakers as to the best production practices involved in preparing cream for churning. These differences are not based upon the whims or caprices of individual buttermakers, for seasonal and sectional influences make it necessary in processing different cream supplies to vary and adjust the degree and type of production practice. At least 75 per cent of the creamery butter produced in the United States is made in sections of diversified agriculture; whereas, the areas in which climatic conditions and density of dairy development contribute most to relative ease in the production of the highest grades of butter contribute only 25 per cent. The skill of the artisan is still, therefore, a vital factor.

Empirical methods play an important part not only in the manufacture of butter but in the practice of scoring or grading butter for market. Butter has been graded for years by the skilled taste sense of commercial and official judges on the arbitrary and numerical basis of 45 points (out of 100) for flavor, 25 for body, 15 for salt, 10 for color, and 5 for appearance of package. Such procedure of rating merits and defects in the factors mentioned might be criticized because it does not take the judgment of butter quality out of the field of human error. On the other hand, it will be granted that in judging the quality of food products, the consumer is guided by the character of its taste, aroma, and appearance as well as by its sales price. Thus in merchandising its product, the butter industry is confronted with consumer preferences which it cannot afford to ignore in passing judgment upon the results of its own labor.

Inherently, butter is one of the best keeping food products known. In milk, germs have the most ideal conditions for their destructive work, which is hastened by the fact that they are readily distributed throughout the entire liquid mass.

Unlike fruits and vegetables, butter is neither a living tissue nor has it any natural protective membrane or skin. Furthermore, bacteria are distributed throughout its mass as they are in milk, yet butter does not spoil nearly as readily as milk. This is not due to advances of science alone for there were buttermakers who produced butter that kept for several months even before pasteurization of the cream was practiced or artificial refrigeration was known. It appears that bacteria do not ordinarily cause the ready spoilage of butter, although there are certain types of bacteria that can be relatively swift in their deterioration of butter. Such infections, however, are infrequent and constitute the exception rather than the rule. Pasteurization of the milk or cream from which butter is churned will, of course, destroy virtually all the microorganisms which might impair its keeping quality. Normally, the microorganisms with which we need be concerned are those which infect the cream or butter after the preliminary pasteurization. Relatively, ordinary bacterial infection is not a serious factor in the spoilage of butter. Considering the facts that present methods of buttermaking do not exclude the possibilities of some bacterial infection, that butter is not sterilized nor ordinarily kept in air-tight containers, that butter is marketed in wooden tubs or individual packages all without any special aseptic precautions, it is worthy of note that butter does truly possess superior keeping quality. In this connection, Rahn's explanation of the role bacteria play in the keeping quality of butter cannot be disregarded:

"The reason for the comparatively good keeping qualities of butter is its structure. Butter consists of at least 80 per cent fat, and not more than 16 per cent moisture, the remainder being salt and curd. The moisture consists of the buttermilk remaining in the butter from the churning and of water from the washing of the butter immediately after churning. It is distributed in the butter in small droplets, the smallest ones being smaller than the fat globules of milk. These droplets have been . . . counted and measured by Boysen, and he found that butter contained between 10,000,000,000 and 20,000,000,000 droplets¹ per gram of butter, most of the droplets being less than 5 microns in diameter.

"This distribution of the moisture in butter accounts for its good keeping. The largest number of bacteria in butter ever recorded in the literature is 57,000,000 per gram. If this number is compared with the number of moisture droplets, it becomes evident that there are not nearly enough bacteria to supply every moisture droplet. Even with the most uniform distribution, only one droplet out of two hundred

¹ 4½ to 9 trillions of droplets per pound of butter.

can contain a bacterium, and the other 199 droplets must be free from bacteria. A computation on the basis of the averages of Boysen's counts and measurements shows that of the total moisture in butter about 99.0 per cent must be free of bacteria if the butter contains 10,000 microorganisms per gram, and that even with 10,000,000 per gram, more than half of the moisture is free from bacteria.

"It is very interesting to speculate as to how this would affect spoilage . . . the moisture droplets in butter are nothing but spaces between the fat globules and since the fat globules of the cream have retained their membranes, these membranes of hydrated colloid form a connection between all moisture droplets so that the hydrated colloid is the continuous phase, and each fat globule is isolated from the other. . . ."

Because of its retarding influence upon microbial development, salt has been credited with the improved keeping quality of butter, but salt also greatly increases its chances of chemical deterioration. Salted butter, for example, occasionally develops a fishy flavor-defect in cold storage in which its acidity and metallic contamination are involved. In recent years, however, it has been well established that the development of fishy flavor in salted butter can be minimized, if not avoided, by proper control of its acidity. Gilmour and Arup, in their studies, concluded that "a tentative pH Standard figure of not less than 6.7 is suggested for butters to be sold stored." Loftus-Hills, Scharp, and Bellair obtained very similar results with Victorian salted butter while Hussong and Hammer have observed that creamery butter produced in the Middle West and having acidities varying from pH 6.8 to 7.4 kept better, whereas more acid butter frequently developed fishiness.

While fishy flavor is one of the major defects which can occur in cold-storage butter, there are other off-flavor developments. Proteolytic bacteria can propagate and develop serious defects in butter during storage, particularly with low acidity or a high pH value, for example, pH 6.8 to 7.4, although butter of higher acidities sometimes develops similar taints. Low acid butter, while not possessed of a relatively high flavor score, will retain its flavor score in cold storage more consistently than the more highly flavored, more acid butter. This is confirmed by the trade experience that good-quality centralized butter made from sour cream can be withdrawn from cold storage with less loss of score than ripened sweet-cream, high-scoring butter.

It is known also that cheesy defects sometimes develop in butter, and generally the proteolytic activity of microorganisms is considered responsible. Actually, lipolysis is often involved with the formation

of caprylic as well as butyric acid. At best, the knowledge of the chemical deterioration of butter is largely empirical.

While chemistry and bacteriology have contributed to the knowledge of butter deterioration, they have likewise played a beneficent role in the technological exploration of the art of buttermaking. We recognize that the palatability of butter is due in large measure to its "fatty," or perhaps "creamy," taste sensation. Its fragrance, however, involves some more subtle flavor or aroma.

As early as 1850, buttermakers learned that the flavor of butter could be definitely influenced and improved by the acid fermentation, or "ripening," of the cream from which it was churned. A vast number of scientists have made valuable contributions to this subject, but discoveries in 1919 by Hammer and Bailey in the United States and by Boekhout and deVries in Holland proved that the desirable flavors in butter cultures depended upon at least two different types of bacteria. In 1929, ten years later, van Niel, Kluuyver, and Derr in Holland were studying propionic acid-fermentation processes and discovered that diacetyl was responsible for the characteristic aroma of fine butter. They found from two to four parts per hundred thousand of diacetyl in fine butter; and when these concentrations were added to a butter neutral in flavor, there was developed a flavor and odor that was unmistakably similar to that of good butter. They concluded that diacetyl is either responsible for the aroma of butter or is the principal component of the aromatic ingredients. They also later found that the bacteria causing fermentation of the citrates in milk were responsible for the production of diacetyl and associated neutral compounds.

The modern conception of the mechanism of aroma production in butter cultures is approximately as follows: The *Streptococcus lactis* grows rapidly at first, serving to discourage the development of contaminants but primarily to produce lactic acid. This production of lactic acid lowers the pH to approximately 4.3, at which acidity citrates normally present in milk are attacked by the citrate-fermenting bacteria which is also present in the butter culture, or "starter," as the buttermaker calls it. As soon as fermentation of the citrates begins, appreciable increases in the amount of acetylmethylcarbinol are produced. Concurrently, there is a development of volatile acids, principally acetic and some propionic. Acetylmethylcarbinol appears to be the first neutral compound produced by the citrate-fermenting bacteria, but there is then presented the possibility of oxidizing some of the carbinol to diacetyl or of reducing it to 2,3-butylene glycol. An oxidation is evidently the preferred change since it yields diacetyl, which is the compound having the desired aroma; while the reduction

yields the glycol, which is probably never oxidized to diacetyl in a butter culture under practical conditions.

The chemical changes which take place in the ripening or souring of a butter culture, according to Hammer, are as follows:

Non-volatile acid	Neutral compounds
Lactic	Diacetyl
Volatile acid	Acetylmethylcarbinol
Acetic	2,3-butylene glycol
Propionic	Protein decomposition products
Gas	Amino acids and also more
Carbon dioxide	complex compounds.

Probably this list does not include all of the chemical compounds produced and additional research may extend the number.

In the proper handling of butter cultures it is highly important to know that the microorganisms are actually functioning in active production of acetylmethylcarbinol and diacetyl. Starters which have become weak in flavor almost invariably are found to have degenerated into simply a culture of *Streptococcus lactis* in which the *Streptococcus citrovorus* and *Streptococcus paracitrovorus* have become diminished in numbers to the point where their citrate-fermenting properties have become seriously impaired. Although the loss of flavor can be readily detected by one skilled in the art, it is advisable frequently to keep more accurate check on butter cultures by chemical analysis. Many butter manufacturers follow the practice of analyzing their butter cultures periodically.

This fact led to the very practical suggestion of Ruehe and Ramsey to utilize starter distillate for imparting to butter a fine flavor. These investigators found that a well-ripened butter culture rich in its acetylmethylcarbinol and diacetyl as well as its volatile acidity constituents could be steam-distilled so as to recover these essential flavor ingredients in the condensed distillate. Furthermore, it was found that the direct addition of such distillate to the butter itself produced a result which is indistinguishable from butter produced from the same cream previously ripened with the butter culture itself. Obviously, such a method of flavoring butter has many advantages not possessed by the practice of using butter cultures for ripening the cream before churning. In the first place, there is no certain means for controlling the production of flavor in a butter culture *per se* and consequently, its standardization in the resulting butter is impossible. On the other hand, the butter-culture distillate can be analyzed and standardized in its flavor constituents with the result that its incorporation in butter can be definitely controlled. The possibility of con-

taminating microorganisms influencing the quality of butter is eliminated in the use of butter culture distillate, a certainty which does not always exist when butter cultures are used for ripening cream to be churned into butter. This discovery by Ruehe and Ramsey promises to be an important development in the art of buttermaking because it permits the more precise control of flavor in butter.

Canning of Cheddar Cheese. L. A. ROGERS, Bureau Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

By "Cheddar cheese" is meant the ordinary variety made very extensively in the United States, which is a modification of the English Cheddar. It is hardly necessary to call attention to the handicap put on this cheese, which when properly made is a very excellent cheese, by the type of package in which it is marketed. This package has the disadvantage of very considerable shrinkage owing to evaporation and mechanical injuries in handling; in an aged cheese there is also a great deal of waste in the cutting. Furthermore, as sold in the grocery or food shop it makes a rather unattractive package for the consumer. A still further disadvantage is that there is no practical way of branding it so that the name of the manufacturer goes with the package. Consequently the manufacturer has not the incentive to make a high-grade product that he has for some other dairy products.

Various attempts have been made to solve the problem. The most successful one thus far is processed cheese, by which the natural cheese is mixed with water and an emulsifier and heated to make a homogeneous mass. This can be molded into any suitable package and can be wrapped so that it does not mold. So far as the package goes, it is entirely satisfactory. The process, however, has the disadvantage of destroying the original flavor to a very large extent, so that the characteristic Cheddar flavor is lost.

The difficulty of packaging the natural cheese in a suitable consumer package comes from its tendency to mold and the difficulty of wrapping the cheese in any way so that air can be excluded and the mold prevented. This latter difficulty is due to the fact that the natural cheese, particularly in the early days of its ripening, develops a very considerable amount of gas, mostly CO_2 . The pressure developed will crack or break any impervious package.

Various attempts have been made to can Cheddar cheese in its natural condition, but the formation of gas is so great that sooner or later the sealed cans will swell. Of course a swelled can is unsalable. Otherwise the package is satisfactory.

We have attempted to solve that problem by the use of a can fitted with a one-way valve which permits the escape of the gas formed in the cheese ripening but does not admit any air. The valve we have used was designed and patented by one of the canning companies, but there are, I believe, a number of valves that could be used. In packaging this cheese we press it into a cylindrical form, perhaps 12 or 15 inches long, and then cut it to the proper size for the can. Of course almost any size can be used, depending on the marketing requirements; for example, a 12-ounce can is used quite extensively on the Pacific Coast for a consumer package and is satisfactory for this purpose. The cheese is wrapped in cellophane, foil, or parchment, put into the can at once as it comes from the press, and sealed. Then it is put away in the curing room and requires no more attention until ready to be sold.

One of the chain stores uses a five-pound can in which is packed ten half-pound prints. The dealer opens the can and the cheese is ready for dispensing in half-pounds.

One of the difficulties encountered in any packaging of this kind is the tendency for dealers or consumers to put the package on the shelves at ordinary room temperature. If the temperature gets too high, there is apt to be a leakage of fat from the cheese, which gives it a rather unsightly appearance. This has been largely overcome by a method which is a combination of pasteurization and homogenization. One of the ten-pound cans thus treated was carried by automobile in the hottest weather of the summer from Washington to a camp in Maine and came through without any greasiness whatever. The pasteurization retards the ripening; therefore, if pasteurized milk is used, it is necessary to ripen the cheese for a longer period.

Obviously this is not a procedure to use with poor cheese. The canning process in itself has no effect on the ripening, except that since there is no evaporation there is no loss of moisture and the canned cheese comes out a little higher in moisture, may ripen a little faster, and be a little softer. If one puts poor curd into the can, however, he will get poor cheese out of it. Consequently, it is necessary to be very careful about the quality of the cheese. That is largely a question of quality of the milk, control of the acidity, ripening temperature, and various factors of that kind.

With this canned product there is no shrinkage, no loss in cutting the cheese, no loss from rind nor from the crumbling that always takes place when one cuts a well-ripened cheese. Of course the canning adds to the cost. For this type of cheese, in a 12-ounce package, there is an additional cost of perhaps five cents a pound at the present price of the cans. That figure is based on a small production. On a

larger production this cost could be reduced somewhat, and with the larger can the cost is proportionately less per pound. Partly to offset that, there is the elimination of shrinkage, the cost of handling the cheese in the curing room, the elimination of loss in cutting the cheese, and various other items. This type of canned cheese could be sold for about the same price per pound as any other aged cheese which usually sells for around 35 cents a pound. One may retail the 12-ounce cans profitably for 30 or even for 25 cents a can if handled on a large enough scale.

One cannot yet state how fully this process has come or will come into general use, but by the end of 1937 there will have been something like 700,000 pounds of Cheddar cheese put up in cans. Most of it will be in five-pound cans, but a considerable quantity will be in 12-ounce cans and some in two-pound cans.

Recent Advances in Brewing Technology. GEORGE B. SIPPEL, The Burger Brewing Company, Cincinnati, Ohio.

Brewing is a highly complex process employing almost every known phase of chemistry and physics. In addition it demands of agriculture the furnishing of basic raw materials of definite specifications. American beers have specific properties which require the development of special varieties of six-rowed pedigreed barleys as distinguished from the two-rowed pedigreed barleys of Europe. There are a few two-rowed types of barley in use in California. The U. S. Department of Agriculture, Bureau of Plant Industry, and the University of Wisconsin, Agronomy Department are working intensively on this particular problem.

Malting has become a specialized industry confined to a few large breweries and to commercial malting establishments.

A recent innovation has been the malting of barley in vacuum driers. Malts prepared in vacuum drums possess an unusually high enzyme content; in some instances the diastatic power runs well over 200°, whereas standard brewers' malts test about 90° to 130° Lintner.

HOP CULTURE

In former years hops were judged as to brewing value solely by physical examination, appearance, color, aroma, freedom from leaves and stems. Today the chemical analysis is assuming considerable importance, studies on the transition of alpha resin or humulin to beta resin or lupulin are receiving increasing attention. Wollmer has recommended an evaluation factor to establish the brewing value of hops of alpha times beta resins divided by nine.

The antiseptic value of hops and their ability to inhibit bacterial growth has been known but it is only recently that we have had defi-

nite proof of this interesting property. Walker, Hastings, and Farrar (1932) established an antiseptic value of the alpha resin or humulon at 400 times that of phenol using a pure-culture bacillus as an indicator. Shimwell and others, however, have definitely established the fact that this antiseptic power applies only to gram-positive organisms. Gram-negative organisms are not affected.

Oxidation prevention is vitally important in preserving hops. Hops are harvested in August and September. They are used in brewing in the succeeding ten months. A rapid decline in flavor and bitter value will result if hops are not stored in dark, cold storage below 4.4°C. (40°F.).

BREWING RESEARCH

Studies on the behavior of selective enzymes, pH, and rH are popular research problems of modern brewing technology; however, much could be accomplished if we had a better knowledge of starch products. Zhea Maiz is still the one and only source of corn starch for breweries but Zhea Maiz green from the fields, Zhea Mais air dried, and Zhea Maiz kiln dried react quite differently as regards their susceptibility to the action of amylolytic enzymes.

We have a similar problem in the use of rice. East India, Siam, California and southern rices possess varietal characteristics not fully understood.

Sorenson's discovery that active acidity is of more importance than total acidity has aided materially in explaining and controlling the complex mashing process in brewing. It has helped to explain the different behavior of brews mashed with carbonate waters and those brewed with waters containing sulphates and chlorides. It has established the fact that brewing water should not be alkaline, a pH of 6.8 to 7.0 being preferable, and clears up the question as to the beneficial presence of calcium sulphate and sodium chloride in certain brewing waters.

Sherman and Thomas (1920) in Studies of the Amylases established the optimum pH for pure diastase at 4.2; however it is interesting to note that in a heavily buffered substrate, such as brewers' wort, the optimum pH for diastase is 5.1 to 5.3. We have two amylolytic enzymes to deal with; one, the amyloclastic or starch splitting, the other, the saccharogenic or sugar forming. Brews which are mashed in at a pH below 5.0 result in poor conversion and starch turbidity. It is reasonable to assume that the saccharogenic enzyme may have an optimum pH of 4.2 but the amyloclastic requires a higher pH. Both enzymes work in perfect harmony at pH 5.2 to 5.4.

Proteolytic activity is not equally sensitive to pH, higher yields of soluble proteins being obtained at a pH as low as 3.7. Adler places

the optimum for proteolytic enzymes at 4.3 to 5.0. Lundin found a protease with an optimum activity of 3.7 to 4.3 and a pepsinase with a maximum activity of 6.1 to 6.4 which liberates amino acids from polypeptides.

The enzyme phosphatase responsible for the modification of organic phosphates into inorganic phosphates has an optimum pH of 5.2 to 5.3 according to Lüers and Silbereisen. The inorganic phosphates together with the amino acids and organic acids present (lactic acid) help to establish the pH value of the mash. According to the investigation of Dufour the buffer substances present in the substrate of the mash are extracted during the initial stages of the mashing.

PROTEIN PROBLEMS

Malt contains a complexity of high and low molecular proteins; some go into complete solution, some go into solution as border-line colloids, some precipitate as coarse or fine particles. The reaction of the tannins released from the husk of the barley malt and from hops with these proteins play a vital part in the production of chill-proof beers. According to Hopkins, protein-tannin compounds are formed rapidly at high temperatures under violent movement and immensity of surface, whereas low temperatures facilitate its separation from solution and surface activity facilitates its separation into coarse flakes. At the pH of beer 4.4 to 4.6 we always have present positively charged proteins capable of combining with tannins and consequently we always have the problem of haze formation.

The American Brewing scientists, Max and Leo Wallerstein, conceived the idea of adding selective proteolytic enzymes to beer immediately after the fermentation period to work upon these protein structures and thereby succeeded in delaying the formation of protein tannin haze substances. This simple treatment has made possible the production of haze-free, chill-proof beers.

The study of the protein-tannin complex is today one of the most active problems in modern brewing research. Not only is the selection of the proper raw materials involved, but recent studies of Seligman, Clendinnen, Bishop, Noritz, and Watkins indicate that the rate of cooling brewers' wort between certain temperatures—60 to 21.1°C. (140 to 70°F.)—has a direct bearing on precipitation of protein tannins. This rate varies with the raw materials but it has been established that a difference in the temperature rate of cooling will materially alter the rate of clarification. Clendinnen suggested a yardstick of measurement for this phenomenon, terming it "the critical break rate." The work is comparatively new and although it has been demonstrated that quicker cooling rates produce brighter worts, their effect on flavor has not been established.

FOAM FORMATION

A creamy, compact head of foam, so desirable on a glass of beer, presents an interesting story. Foam has been described as a system of gas bubbles dispersed in a liquid. Actually, however, foam always gathers on a liquid. It is well to remember however, that foam will never form on a true liquid. Froth will only form on molecular or colloidal solutions. Surface tension plays a vital role in foam formation, as does the law of Gibbs and Thomson—"In any solution the substance with the lower surface tension is concentrated on the surface." Viscosity plays an important role.

According to Jakob Blom, "in considering foam on beer we must distinguish between head formation and head retention." Head retention in beer depends on three principal properties:

- a. The presence of easily coagulable surface-active colloidal substances.
- b. A low surface tension (44 to 50 Dynes).
- c. High viscosity (Dextrin).

Surface-active substances in beer are alcohol, volatile acids and esters, colloiddally dissolved proteins, and hop resins. These give beer its low surface tension—44 to 50 Dynes—resulting in the formation of smaller diameter bubbles than would be formed under similar circumstances in water.

Lüers has calculated that 100 c.c. of carbon dioxide liberated by pouring one liter of beer into a glass will form 3,000,000 foam bubbles with a diameter of .4 mm. and a surface of 1.5 sq. cm. This surface is 144 times greater than one single spherical bubble of 100 c.c.

One great problem of the brewer is to educate the purveyor of beer to properly cleanse service glasses. Beer poured into greasy glasses or glasses which previously contained milk or remnants of fat or my-lady's lipstick will not retain a head of foam. Fats or soap have great surface activity and will expel the proteins from the surface. At the low pH of beer the fats are hydrolized to free fatty acids with the result that the foam collapses. Glasses should be cleansed with alkaline detergents—never with soap.

The significance of oxidation-reduction potential in brewing is receiving impetus through the excellent work of De Clerck, Mendlick, Hartong, Van Laer in Europe, and through the work of Laufer in America.

Oxidation-reduction reactions take place in every phase of malting and brewing. From a practical standpoint it is receiving wide attention in the elimination of air in the packaging of beer, whether barrel, bottle, or can.

Bottled or canned beers containing less than 2.5 c.c. of air per 12-ounce package resist flavor changes and haze formation far more readily than higher percentages. What we need are simple, reliable methods to measure these interesting phenomena.

BREWERY CONSTRUCTION AND EQUIPMENT

Brewery construction and equipment has kept pace with advancement in brewery science. Modern breweries no longer are bound to the gravity-flow system.

Steel storage tanks, either glass-enameled or coated with wax or bituminous plastic compounds, are replacing wood. They are supported directly by the building structure columns and are built up in tiers, access being gained by catwalks in place of floors. Where plastic coatings are employed the composition of the steel used in fabricating the tanks is important. This should be flanged steel of a low ductility and of the following analysis:

Carbon.....	0.22%	Phosphorus.....	0.012%
Manganese.....	0.39%	Sulphur.....	0.030%

Supporting steel tanks directly on the H columns and members of the building construction raises the question of deflection. In one instance this has been less than $\frac{1}{16}$ of an inch.

Four-point support of steel tanks also permits the construction of buildings with floor systems calculated only to carry light live loads as the heavy loads are conveyed directly into the steel members and columns. Multiple-support tanks require designing of floor systems conveying live loads of 500 pounds per square foot whereas four-point suspension requires floor loads of 100 pounds or less, resulting in considerable saving in building construction costs, particularly with the high cost of labor and materials.

Several brewers are using concrete fermenting and storage tanks lined with a substance similar to Bakelite. The one weakness of concrete storage tanks is their failure to withstand pressure in excess of 10 pounds.

Special Problems in Fish Canning. M. P. VUCASSOVICH, Gorton-Pew Fisheries Co., Ltd., Gloucester, Massachusetts.

In dealing with food technology we are concerned with national resources—the very foundation of American strength. No matter what sociological and economic troubles beset us, no matter how and when they are solved, they remain incidental to the fact that the people must look to the land and to the sea for their food supplies.

One of these two sources of our nation's wealth, the sea, produces food in variety and abundance and the fisheries represent one of our

great food industries. The fisheries industry is a big business and is conducted as a big business. According to the United States Bureau of Fisheries, our commercial fisheries provide employment for over 204,000 persons, of whom 120,000 are fishermen. In addition to these, about 300,000 persons are engaged in allied industries, manufacturing products or things used by the fisheries. In general, about one out of every 200 persons in this country is directly or indirectly interested in the economic welfare and prosperity of our commercial fisheries. The annual harvest of these fisheries amounts to about three billion pounds of protein and mineralized food.

It is not necessary to dwell here upon the nutritive value of our marine products. In general, they have been found to be good sources of vitamins, minerals in quantity and variety, and proteins of high quality. Rather I want to present some special problems in fish canning.

For many generations the preserving of fish has been, and is, done by drying in the sun, salting dry or in brine, pickling in vinegar, and cooking and keeping in oil. While these methods are practical, nevertheless they have a tendency to alter the taste of the fish.

It is of importance to preserve articles, either as nearly as possible in their original state or in some other in which, though their form and properties be altered, their nutritive powers are retained.

Canning of fish results in the making of better and safer foods available at all times and places, bringing this commodity to consumers living far from the source of supply, where certain salts, such as iodine, may be lacking in their local fare. Present canning methods are the result of practice and scientific studies, combined with engineering skill to handle these products economically and practically.

One of the most important problems we have in canning is to please the eye of the buyer as well as his palate. Thanks to the work of eminent scientists and to research in our laboratories, we do not have to worry about keeping qualities. The appeal to the palate is not one that can be ignored. Advertising and merchandising efforts are largely centered upon this appeal to taste and flavor, but another important factor is that of quality.

One of the problems encountered when the sanitary can came into use, for example, was darkening of clams in the can. For a while we were puzzled, but the trouble was traced to the sanitary can being free in its inside seam from any acid flux. By using an acid brine in the can—about .1 per cent of acetic acid—this trouble was eliminated. This method of prevention was devised by S. C. Prescott about 1898, but had never been published. C enamel does not eliminate the darkening of clams.

Canadian packers rinse lobster meat in a weak citric acid solution, which is effective in preventing darkening in processing. Plain cans are preferable to C enamel cans, since it is claimed that a slight foreign flavor is observed in lobster when packed in enameled cans.

The fish canner must also be ready to attempt new products. A product with which we have had a particularly interesting experience is Ready-to-Fry Codfish Cakes. Needless to say, probably one of the greatest uses for salt codfish is for fish cakes or fish balls. This original New England delicacy is a popular item on the bill of fare in almost every part of the United States today. The popularity of the codfish cake led us to develop a fully prepared fish cake. The old-fashioned method of making codfish cakes necessitated the soaking and picking of the dried fish; boiling, peeling, and mashing of potatoes; then the careful mixing of these two ingredients in the desired proportions. The laborious preparatory processes which consumed so much of the housewife's time were eliminated, with a resulting product that requires no labor except that of shaping and frying. This meant that the serving of codfish cakes could be made a simple and quick matter. Fully aware of the fickleness of the human appetite when appealed to by prepared food, we desired to make this product as perfect as possible before offering it to the public. While the production of this food material on a domestic scale seems simple, although time-consuming, its successful commercialization required that many problems be met. It involved the knowledge of the expert cook as well as the technologist, and the critical opinion of several thousand consumers who were asked to pass judgment on the quality, appearance, and price of the products in the course of the 18 months or more required to bring a satisfactory ultimate result, which has proved a great commercial success.

A similarly careful study of details made possible the production of a canned salt mackerel and canned mackerel fillets for both of which there is a large consumer demand, only limited by the vagaries of the fish itself, for the mackerel catch is one of great uncertainty. On the other hand, the tuna, never before packed in New England, offers a new product for the canner who will apply careful research to his problems. Doubtless there are many other fish products that will yield to such detailed study. The chemist, the bacteriologist, the biologist, and the food technologist are all needed in solving these problems.

Our early founders who began fishing off the New England Coast in 1623 would indeed be puzzled if they could witness the ramifications which their pioneering spirit may rightly be said to have achieved in the fishing industry as it is today.

Better Meals for Tomorrow. LEWIS W. WATERS, Vice President, General Foods Corporation, New York City, New York.

The personnel of this Food Technology Conference is concerned with almost every food material, operates many different food processes, utilizes all types of food containers, and is skilled in every branch of food chemistry. Numbered among you are producers, manufacturers, distributors, transporters, and scientists; and every one here is a consumer who is interested in better meals for tomorrow.

You represent a great industry responsible for some twelve hundred items sold in the modern grocery store to meet the public demand for a widely diversified national menu. Even though prices of foods are kept low by the terrific competition of more than forty thousand manufacturers whose products reach the public through approximately a half million retail outlets, the food bill of this nation is one-fourth of its annual income. You are in industry where monopoly virtually is nonexistent; and competition is intensified by the daily shopping of some twenty million housewives. Each year one and a quarter million new brides start housekeeping.

Many of you are devoting your time to pure research, hunting for facts which may or may not find immediate application but which, contributed to a storehouse of new knowledge, will be available when needed. Others are in commercial research, digging out facts to solve a definite problem and making use of this acquired experience to give us better meals for tomorrow. All of you are eager for new ideas, the exchange of which is the reason for this conference.

New ideas are assets to the food industry as much as raw materials, plants, or containers. We depreciate old plants and equipment and get new ones of higher speed and efficiency. We must replace old, worn-out ideas with new and better ones. The food technologist of today must be in the front rank of development activities. He cannot long be parasitic and depend upon his competitors to show him the way.

The old conception of foods of value was modified by the discovery of vitamins, and it is probable that bigger and better vitamins are yet to be recognized, isolated in pure form, their chemical composition and structure determined, and probably synthesized. The effect of knowledge of vitamins on our diet is well known and is indicated by the enormous increase in consumption of fruits and vegetables and the introduction of old foods in new forms, such as tomato juice, which is now one of the largest items in the canner's pack.

About the turn of the century came the great change in food merchandising through the introduction of packaged foods formerly sold in bulk. On the shelves of the grocery store of today is an array

of colored cartons, bottles, and cans, all demanding your attention and tempting you to buy them.

About a decade ago there came another important change in the food industry—the merging of companies into larger groups. This was done to obtain the advantages of group buying, manufacturing, and selling, and to insure steady employment of the operatives without seasonal fluctuations. The large food companies became publicly owned businesses, numbering thousands of housewives among their stockholders. These were mergers not only of products and businesses but also of brains and experience, affording the opportunity for centralized research by the food technologist. These consolidations brought together various units in the food industry, each skilled in its particular branch of food chemistry. Centralized research meant maximum utilization of the results of scientific research without duplication of effort.

What the food technologist has accomplished in the past forty years is a profoundly impressive story, which has not been told adequately to the consuming public that has most benefited. As the oldest of our industries, the food industry might be expected to be the most conservative. On the contrary, few industries have been so hospitable to scientific development. The manufacturer made the food technologist the keystone of his structure before the product could be marketed.

Products, machinery, plants, merchandising methods, and packages have been allowed to endure only until better ones were created. There has been a constant effort for quality, higher and higher quality, giving the consumer more for his money. The food manufacturer has learned that he can obtain consumer confidence by giving quality at a reasonable price with service. The public eats better foods today. They are more nutritious; they are more delicious; they are more attractive to the eye, because of the work you are doing.

The housewife has learned that packaged foods can be depended upon every time they are used. The kitchen has become a laboratory. Few housewives now cook with a pinch of this or a dash of that. They use a measuring cup and an oven thermometer. Preparing meals has become a science using formulas and recipes prepared by experts, eliminating natural skill but insuring results. The number of schools offering courses in home economics has more than doubled in the past two decades, and registration in these courses has more than trebled in the same period.

All of us at this conference want to see better meals for tomorrow. That is the responsibility for you and me and the millions of us connected with the food industry. The fate of a nation, as well as that

of an individual, may be determined by what that nation eats. There may be a parallel somewhere between the sensational emergence of this country as a world power and industrial empire since the turn of the century, and the improvements in foods during that period. In this part of the world the finest fruits, vegetables, meats, fish, and cereals are available in abundance at such prices that they can be enjoyed by a large and ever-increasing majority of people. Little wonder then that for the most part we are optimistic, ambitious, and cheerful even in the face of tremendous difficulties. That attitude is partly due to our food and diet.

On the other hand, a few other parts of the world are checkerboards of suspicion, of war or war threats, and weary despair. The food technologist with the soundest of reasoning can point out that such discord may be partly dietetic as well as economic and political. Whoever saw any one get up from a good meal with anything but a cheerful, tolerant attitude toward his fellow men? Good food benefits the nervous and emotional system just as much as one's physical nature. Food technologists of all nations may eventually help solve the "jitters" of nationalities by helping to correct the malnutrition and under-nourishment which provokes war. Much of the job, of course, would get into fields of agriculture, economics, education. A generation ago we said, "Food will win the war." Our civilization today would be better served by the slogan, "Correct eating and food in sufficient quantity will help avoid war."

Realizing some of the great responsibilities and opportunities for human service which are ours, we should feel that truly there is no more inspiring job than that in which all of us at this conference are at work—that of assuring the human race better meals for tomorrow.

Research in Metallurgy and Its Significance in Canning. ROBERT S. WILLIAMS, Head of Department of Metallurgy, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Metallurgy is one of the oldest of the sciences. Tubal Cain is mentioned in old poems and stories as a fabricator of metals, and down through the ages the metallurgist has played a vital part in the development of civilization. Up to the turn of the present century, however, the metallurgist was primarily concerned with the production of metals from their ores and was interested but slightly in their properties.

About 1895 Osmond in France, Roberts-Austen in England, Sauveur and Howe in this country, and a few other inquisitive souls began to wonder why steel behaved as it was known to do under the influence of various changes in cooling rate and reheating temper-

atures after rapid cooling. This group of pioneers opened a new field of investigation, and the results of their efforts have led to the development of a somewhat different type of metallurgist who has come to be known as a physical metallurgist, since he is primarily concerned with the physical properties of finished metals and alloys, while his older brother, the production or process metallurgist, is chiefly interested in the chemical processes used in making steel, brass, or other alloys. The physical metallurgist came to be definitely recognized during the World War, and developments in his special field have taken place with ever-increasing momentum since that time. He is a distinctly composite individual who reaches out into all fields of science for such information as may be useful in his special problem.

From his older brother, the process metallurgist, he must learn the effects on properties of metal as influenced by the method of manufacture. From the mechanical engineer he has learned the significance and importance of the testing of materials, since such properties as strength, hardness, and ductility are of major importance. The physicist has shown him how the X-ray may be used not only to detect flaws in his finished product, such as faulty welds, slag streaks, and the like, but also as a method of studying the ultimate structure of metals and alloys to find the arrangement of atoms which in turn influences markedly the grosser characteristics of metal. The physicist has shown him, too, how magnetic forces may be used to give invaluable information with regard to minute defects that can be detected no other way. Finally the chemist has contributed in fullest measure to studies of the corrosion of metals. So great indeed are the chemist's contributions to the corrosion problem that it is a valid question as to whether corrosion is not really a problem for the chemist rather than for the metallurgist. Since, however, the physical metallurgist is vitally concerned with *all* properties of metals there is a growing tendency for corrosion research to be allocated to the metallurgical laboratory. This brief review of the interests of the physical metallurgist indicates some of the lines along which metallurgical research may be significant in the canning industry.

My personal connection with the canning industry is limited to the study of a specific problem in corrosion, so any discussion of other problems must be very general in character. Research in the canning industry, as in any industry in which metals are used, would presumably consist of the following:

1. A study of the properties of commonly used metals with a view to determining their good and bad qualities. As a corollary to this study would be added the development of tests for these properties—tests which are accurate, reproducible, and as rapid as possible. Such

tests require the most painstaking study as they may well become the basis for selection or rejection of large quantities of material involving great sums of money. Research leading to the establishment of such tests may result, and indeed is known to have resulted in the past, in savings amounting to many times the cost of the investigation.

2. The development of new alloys for specific purposes. With more than five thousand different alloys available today it would seem as if this kind of research were not justified, but actually the number of possible combinations of metals is limitless, and alloys will surely be found that are more satisfactory for the canner's needs than those now available.

3. The establishment of suitable standards for acceptance of metals and alloys purchased either for the manufacture of cans or to be used in kettles, trays, pans, or other equipment of the canning industry. These standards may be physical or chemical but probably will be both.

The application of research to a special problem may be illustrated by a brief account of an investigation carried out here at the Massachusetts Institute of Technology in association with a can-manufacturing company. A most serious situation had developed leading to an enormous number of failures owing to corrosion inside their cans, with the corresponding formation of hydrogen. Springers and swells developed to an alarming degree. A detailed investigation of the cause of these failures, involving several million separate observations, was carried on by the company. Studies were made of different varieties of fruit, soil conditions in different orchards, differences in fertilizers and sprays, differences in cooking methods. Hindsight is always more certain than foresight, but it seems now as if a metallurgist familiar with corrosion problems would have felt that differences in the metal from which the cans were made was probably of greater significance than differences in soil conditions. In any event it was not until almost every avenue of approach had been investigated that a careful study of the tin plate was undertaken and the answer found in a relatively short time. Even then some of the tin-plate manufacturers were skeptical of the significance of the test methods developed and had serious doubts as to their validity for evaluating plate.

The question was referred to M.I.T. to decide whether or not the test methods were sound and also if possible to determine in some other way the reasons for the failure. Our observations, made wholly independently on experimental packs prepared under carefully controlled conditions, confirmed the earlier results in every way; and it was obvious that the difficulty was due to a particular method of manufacturing of the steel base. Other factors, such as the thickness of the

tin coating and differences in chemical composition of the steel base, caused minor but measureable variations in the corrosion rate, but the method of production of the steel base had so marked an effect that other variations were insignificant. Details of the metallurgical investigation which followed the corrosion testing would be of no particular interest, but it may be said that a microscopic examination of suitably prepared specimens gave a positive reason for the failures and again checked in all respects with the tests so skillfully devised and carried out by the research chemists of the can company. This research, though time-consuming and costly, paid for itself many times over; it gave an answer to a question for which the right answer was imperative.

This work ended my direct connection with the canning industry, but in closing may I suggest a few lines along which it seems as if metallurgical research would be profitable. Why is a tin can necessary? Tin is costly and is a strategic material of which we have no domestic supply. Is no other material suitable for the preserving of food? Aluminum alloys are certainly possible but at present too costly. Some grades of stainless steel would be quite satisfactory but again are too costly for canning purposes. Would it be possible to produce at a reasonable cost duplex sheets with the corrosion resistant metal rolled into the steel base? Why not use an untinned steel can? Automatic welding methods could surely be developed to make the fabrication simple and solderless. A steel can would be unattractive on the grocer's shelf and would soon rust but could it not be protected by a suitable varnish at a cost less than that of tin? How about an oxide protection of the Parkerizing type? The housewife might object to a black can at first but she could probably be educated. Are all the metals used in the canning operation itself wholly satisfactory? Cannot cookers, steam kettles, screens, pipe lines, and other pieces of equipment be improved?

These thoughts come from a rank outsider and all of them may have been already considered and rejected by those in your industry who are directly concerned. If they have not, it seems to me that metallurgical research of the most fundamental character is of significance in the canning industry.

A paper entitled, "The Development of a New Container—The Beer Can," was also read by the late Mr. W. E. Taylor, Vice-President in Charge of Operations of the American Can Company, New York.

STABILITY OF VITAMIN D IN IRRADIATED EVAPORATED MILK

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Of all the canned foods entering the American dietary today, evaporated milk probably ranks first in importance. The popularity of this concentrated and sterilized product continues to grow, and in the past few years it has been adopted by many pediatricians as the milk of choice in infant feeding. Since the spring of 1934 several brands of evaporated milk, aggregating more than half the amount produced, have been irradiated with ultra-violet light to increase the vitamin D content.

Rice (1930) reported that there is no depreciation in the nutritive value of evaporated milk in its manufacture from raw milk. Since the evaporation process takes place in a vacuum, this may account for the fact that there is little or no destruction of those vitamins which milk is depended upon to supply. However, in view of the fact that certain vitamins are quite labile, it is natural that interest should be centered on the stability of vitamin D in irradiated evaporated milk.

A survey of the literature reveals that the subject of vitamin D stability and preservation refers primarily to vitamin D solutions and cod liver oil. Steenbock, Jones, and Hart (1923) reported that the vitamin D in cod liver oil was stable to saponification. Hart, Steenbock, and Lepkovsky (1925) showed that cod liver oil mixed with ground grains and stored in cans at room temperature retained its calcifying power for at least six months. On the other hand, Norris, Heuser, and Wilgus (1929) found that the storage of cod liver oil in a mixture of ground feeds resulted in a material destruction of the vitamin D when this feed mixture was kept in burlap sacks at room temperatures from 12 to 16 weeks. Payne (1930) states that one per cent of cod liver oil stored in feed for one year retained its antirachitic potency when tested by feeding to Rhode Island Red chicks for eight weeks. Heuser and Norris (1929) observed that the vitamin D of cod liver oil was partially destroyed by exposure to CO₂-free and moisture-free air at the temperature of boiling water for 12 hours and that it was completely destroyed in 48 hours. Reerink and Van Wijk (1934) reported that solutions of vitamin D in the

original solvents *in vacuo* could be stabilized by an addition of 10 to 25 per cent by volume of vegetable or animal oils. Sheehy (1932) studied the stability of vitamin D in cod liver oil and found that in a completely filled and sealed bottle there was no loss in vitamin D potency when exposed to light, kept in a dark cupboard, or kept in a tin-lined container. Shelling (1937) in working with prepared oil-in-water emulsions of viosterol, which were miscible in water and in milk, found that there was a very marked and progressive loss in vitamin D potency in from three to six months when the solutions were kept in an ice box in tightly stoppered bottles. The loss was only slight when the air was replaced by nitrogen.

Since the work on vitamin D solutions and cod liver oil indicated that vitamin D may or may not be stable, depending on the method of preservation, it seemed advisable to run a series of assays over a period of years to determine what actually was the case in irradiated evaporated milk.

EXPERIMENTAL WORK

The milk samples were received from various plants of each of the several companies manufacturing irradiated evaporated milk. Triplicate samples of each batch were obtained; one was assayed at the time of its arrival and the duplicates were stored for future assay. In this series of tests an attempt was made to select, in so far as was possible, milks assaying 135 units per quart when diluted one to one with distilled water. The temperature of the storage room varied from 4.4°C.(40°F.) in the winter to 43.3°C.(110°F.) in the summer, thus simulating a variety of temperature changes as may be encountered in the commercial handling and distribution of this product.

The U.S.P. XI (1936) method of assay for vitamin D was employed with such modifications as to make it suitable for milk assay. At the time of testing, the cans of milk were placed in hot water at 60 to 70°C.(140 to 158°F.) for 15 minutes and thoroughly shaken every five minutes. This seemed advisable in order to assure a homogeneous distribution of the contents. The milk was then diluted one to one with distilled water and fed daily. Five hundred rats were used in this series of assays. The milks were all run at the 135-unit level and evaluated according to the method employed in this laboratory. U.S.P. reference cod liver oil was used as a positive standard throughout the experiment.

DISCUSSION

That the consumer is at all times assured a fresh supply of irradiated evaporated milk is shown by a check-up of the marketing conditions which normally prevail for this commodity. Distributors

ordinarily do not have on hand more than a two-months' supply of irradiated evaporated milk. The average elapsed time, therefore, between packing of the milk and its consumption by the housewife is

TABLE 1
Vitamin D Potencies of Irradiated Evaporated Milk Assayed at Various Intervals

Plant	Time in years	U.S.P.U. per qt. dil. (1:1)	Plant	Time in years	U.S.P.U. per qt. dil. (1:1)
A	0	135+	L	0	135
	1.50	135		0.90	135
	3.25	135		2.70	125-135
B	0	135+	M	0	135
	1.50	135+		0.90	135
	3.25	135+		2.70	135
C	0	135+	N	0	135+
	1.30	135		0.50	135
	3.00	135		2.25	125-135
D	0	135+	O	0	135+
	1.00	135		0.50	135
	2.75	115-120		2.25	135
E	0	135+	P	0	135
	1.00	135		0.90	135
	2.75	135+		2.60	135
F	0	135+	Q	0	135
	1.00	135+		0.90	135
	2.75	135+		2.60	135
G	0	135	R	0	135+
	1.00	135		1.00	135+
	2.75	135		2.75	125-135
H	0	135	S	0	135+
	0.90	125-135		1.00	135
	2.70	125-135		2.75	135+
I	0	135	T	0	135
	0.90	135		0.90	135
	2.70	135		2.70	135
J	0	135	U	0	135+
	1.10	115-125		0.90	135
	2.85	115-125		2.70	135
K	0	135+	V	0	135+
	1.00	135		0.90	135+
	2.75	125-135		2.70	135

from 30 to 90 days, and only under extraordinary conditions would this interval be as great as six months. During shipping and storage the milk is necessarily subjected to a variety of changes in temperature.

Since conditions of preservation and temperature play a very important role in the stability of vitamin D, it is interesting to note that the results indicate that apparently such criteria are fulfilled in irradiated evaporated milk. It is evident from the data (Table 1) that there is little or no loss in vitamin D potency over a period of two to three years. It may be quite fortunate that in the manufacture of irradiated evaporated milk most of the air is removed before the can is sealed. Consequently most of the oxygen is removed and the primary factor which enters into the destruction of vitamin D is greatly reduced. Those few instances where the data show a slight decrease in vitamin D potency compose only a small percentage of the total, and the loss is of an order which might well fall within the limits of error of a biological assay.

CONCLUSION

It is evident from these data that there is little or no loss in the vitamin D potency of irradiated evaporated milk when stored for two to three years under moderate temperature changes. The results show that in no instance was there any loss whatsoever during the first year. Hence the vitamin D potency of irradiated evaporated milk is assured since the milk is consumed well within this period of time.

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A STUDY OF COMPARATIVE METHODS AND MEDIA USED IN MICROBIOLOGICAL EXAMINATION OF CREAMERY BUTTER

I. YEAST AND MOLD COUNTS

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Considerable work done in recent years indicates that yeast and mold counts of butter and related products provide a reliable index of the sanitary efficiency of creamery production practices.

It is a well-established fact based upon scientific investigation and practical experience that the principal cause of high yeast and mold counts in butter is improper cleaning of equipment *including churns*. We state "including churns" advisedly. Inasmuch as the churn is constructed of wood and because it is difficult, if not impossible, to sterilize, the tendency has been to blame it for most of the ills of insanitary creamery practices. Equipment unquestionably is subject to such an indictment, but it applies also to leaky stuffing boxes, imperfect soldered joints, and open seams just as definitely as it applies to churns. Among the sources of mold contamination, for example, Macy and Combs (1932) discovered that the churn was the source in 65 per cent of the creameries studied, while other equipment accounted for 75 per cent of such infections, starter or butter cultures for 40 per cent, wash water for 44 per cent, and salt for 33 per cent.

In view of the practically universal acceptance of consistently low yeast and mold counts (or their absence) as indicative of proper pasteurization of the cream and effective creamery sanitation as well as satisfactory handling and packaging of the finished butter, this study was made in order to investigate several different methods and media used for such microbiological examination of butter and related products.

We take pleasure, therefore, in reporting some of the more representative results obtained in this study upon yeast and mold counts in the hope that further investigation will be stimulated. While it is our belief that these results have some practical significance, inasmuch as they were obtained from samples of butter produced under a variety of commercial conditions, it still remains the lot of the

scientific investigator to seek fuller knowledge as well as to establish their factual basis.

EXPERIMENTAL PROCEDURE

In 1933 the Committee on Bacteriological Methods of the American Dairy Science Association published a report on methods of analyzing dairy products in which the use of potato-dextrose agar was recommended for making yeast and mold counts. Dilutions were prepared by using 1 ml. and .1 ml. of melted butter directly as well as using dilutions of 1 ml. of melted butter in 99 ml. of sterile water when higher dilutions were necessary. Later, Parfitt (1937) advocated the method of transferring 11 ml. of melted butter into a 99 ml. sterile water blank and using 5 ml., 1 ml., and .1 ml. of this 1:10 dilution to give dilutions of 1:2, 1:10, and 1:100 respectively.

Six different culture media which have been suggested and used at various times for determining the yeast and mold counts of butter were employed in this study and were applied to both salted and unsalted butters. The yeast and mold plate counts of both salted and unsalted butter were determined from duplicate plates prepared from 1 ml. of melted butter plated directly in addition to plates prepared from 5 ml., 1 ml., and .1 ml. of the 1:10 dilution as advocated by Parfitt. All samples of butter were prepared by first placing them in a water bath having a temperature of 40 to 45°C. (104 to 113°F.). After 15 minutes had elapsed, they were shaken until they had a thin, creamy consistency. Dilutions and plating followed immediately, using six different culture media.

The media used in this study were as follows:

1. Freshly prepared potato-dextrose agar
2. Dehydrated potato-dextrose agar
3. Dehydrated peptonized-milk agar
4. Dehydrated malt agar
5. Dehydrated whey agar
6. Dehydrated wort agar.

The dehydrated media listed above were obtained from the Digestive Ferments Company of Detroit, Michigan. The freshly prepared potato-dextrose agar was made in our laboratory at Chicago as needed.

Two hundred grams of potato, previously peeled and sliced, were placed in 1,000 c.c. of distilled water and allowed to boil for one hour. This infusion was then strained through a double thickness of cheese-cloth and restored to the original volume. Twenty grams of commercial dextrose and 15 grams of agar-agar were then added and dissolved by heating in an autoclave at a pressure of 15 pounds for 20 minutes. The agar was then dispensed into flasks in 100-c.c. quantities and sterilized at 15 pounds pressure for 30 minutes.

To the melted sterile agar enough sterile tartaric acid solution (10 grams U.S.P. tartaric acid dissolved in 100 c.c. of distilled water) was added to give a reaction of pH 3.5. The amount of the tartaric acid solution was determined for each batch by the electrometric pH method. Generally about 1.9 c.c. of the sterile tartaric acid solution was added to a 100-c.c. quantity of melted agar and shaken well to mix thoroughly the agar and acid. From this a small portion was poured into the sample cup of the pH apparatus which contained a small amount of quinhydrone. The calomel cell and platinum electrode were then introduced into the sample cup and reading was made upon the potentiometer. Correction was made by varying the amounts of acid until the pH of 3.5 was reached. This usually was reached between the addition of 1.9 c.c. and 2.5 c.c. of acid.

Adjustment of the agar was made only for the amount of agar to be used immediately for plating.

With the exception of the preliminary group of counts, in which microscopic examination was used to identify the bacterial colonies, the mold colonies were identified by their profuse growth of hyphae or long filaments; and the yeast colonies were identified by smooth surface colonies that were moist and somewhat raised. All colonies which were not definitely mold colonies were counted as yeasts.

In the first group of samples plated the freshly prepared potato-dextrose agar was adjusted to pH 3.5 while the dehydrated media had the following pH values: potato dextrose, 5.6; malt, 3.5; peptonized milk 6.5; whey, 6.6; and wort, 4.7. The pH values were those suggested by the Digestive Ferments Company. All plates counted in this first group of samples as well as those included in this entire study were incubated for a period of five days at 21°C. (69.8°F.). Although colonies generally began to appear at 48 hours, it was found that better results were obtained by incubating the plates for five days.

Data obtained from the first group of samples plated show that the higher pH values, especially those above pH 4.7, gave high counts of bacterial colonies that were confusing factors in counting the yeast and mold colonies (Table 1). All colonies were identified by resorting to microscopic examinations. While the yeast and mold counts of the butter samples in this first group were not inordinately high from the standpoint of counting, the presence of the bacterial colonies made the task of counting the yeast and mold colonies a tedious one indeed. It was obvious from results obtained in this preliminary group of counts that in making yeast and mold determinations the reaction of the culture media would have to be such that the growth of bacterial colonies could be inhibited to prevent confusion. Follow-

ing this preliminary group of counts, therefore, all media were adjusted to the same pH, namely 3.5.

Fifteen individual samples each of salted and unsalted butter were included in this study extending over a period of six months. Forty-

TABLE 1
*Comparative Yeast and Mold Counts of Salted and Unsalted Butter
Plated on Various Media of Varied pH Values*

Media	pH	1 ml direct		1:2 dilution		1:10 dilution		Remarks
		Yeast	Mold	Yeast	Mold	Yeast	Mold	
Salted butter								
Potato dextrose..... (freshly prepared)	3.5	102	7	190	10	250	0
		81	5	202	10	40	100	
Potato dextrose..... (dehydrated)	5.6	112	4	178	10	220	30	Many bacte- rial colonies.
		92	11	190	10	200	20	
Malt.....	3.5	88	5	80	3	50	10
		63	2	92	4	60	10	
Peptonized milk.....	6.5	150	20	Many bacte- rial colonies.
		76	8	110	30	
Whey.....	6.6	98	22	140	20	Many bacte- rial colonies.
		79	4	68	14	110	30	
Wort.....	4.7	68	8	158	12	190	70
		70	12	140	8	240	30	
Unsalted butter								
Potato dextrose..... (freshly prepared)	3.5	40	5	22	2	80	0
		23	1	23	10	50	20	
Potato dextrose..... (dehydrated)	5.6	36	2	26	12	120	30	Many bacte- rial colonies.
		35	2	38	2	140	20	
Malt.....	3.5	16	3	6	0	20	0
		13	2	12	2	0	10	
Peptonized milk.....	6.5	30	0	14	10	0	80	Many bacte- rial colonies.
		31	0	18	10	40	40	
Whey.....	6.6	17	2	14	4	0	80	Many bacte- rial colonies.
		22	0	18	2	40	80	
Wort.....	4.7	27	13	26	2	30	30
		24	3	30	12	60	30	

eight plates were plated and counted for each sample examined. Approximately 1,000 individual plates were thus counted for their yeast and mold colonies, the data being collected in 30 different tables. In presenting results it was decided to select representative data; therefore, the tables in this paper give the actual data obtained with both

salted and unsalted butters having low, intermediate, and high combined yeast and mold counts. Low counts were considered to be those varying from zero to 30 colonies per ml., intermediate counts those varying between 30 and 50 colonies per ml., while high counts were considered to be those in excess of 75 to 100 colonies per ml. of melted butter.

Data obtained in plating a sample of unsalted butter which was practically devoid of yeast and mold infection, although the potato-dextrose agars and the whey agar indicated a tendency for slight

TABLE 2
*Comparative Yeast and Mold Counts of Unsalted Butter
Practically Devoid of Contamination*

Media	pH	1 ml. direct		1:2 dilution		1:10 dilution		1:100 dilution	
		Yeast	Mold	Yeast	Mold	Yeast	Mold	Yeast	Mold
Potato dextrose.....	3.5	0	0	0	0	0	0	0	0
		0	0	0	2	0	0	0	100
Potato dextrose.....	3.5	0	0	0	0	0	0	0	0
		0	0	0	2	0	0	0	0
Malt.....	3.5	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0
Peptonized milk.....	3.5	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0
Whey.....	3.5	1	0	4	0	0	0	0	0
		0	0	0	4	0	0	0	0
Wort.....	3.5	0	0	0	0	0	0	0	0
		0	1	0	0	0	0	0	0

growths in the 1:2 dilutions (that is, the 5 ml. platings of the 1:10 dilutions), are given (Table 2).

Some very interesting data (Tables 3 and 4) represent yeast and mold counts made upon commercial churnings of butter prepared from the same cream in the same churns before and after the butter was salted. These butters can be considered generally as low-count butters since they range in counts from zero to 30 colonies per ml. The data indicate that the unsalted butters exhibited a tendency to give higher counts from duplicate plates of the 1:2 dilutions (5 ml. of the 1:10 dilutions) than was usually the case when 1 ml. of the melted butter was plated directly (Table 3). Generally the consistency of the duplicate counts appeared to be slightly better where the samples were plated directly using 1 ml. of the melted butter than was the case where the 1:2 dilutions were used. From the data pre-

TABLE 3
*Comparative Yeast and Mold Counts of Unsalted Butters Plated on
 Various Culture Media Adjusted to pH 3.5*

Media	Sample No	1 ml. direct		1 : 2 dilution		1 : 10 dilution	
		Yeast	Mold	Yeast	Mold	Yeast	Mold
Potato dextrose..... (freshly prepared)	3	10	0	12	0	10	0
		8	0	6	0	0	0
	4	2	0	4	0	0	0
		2	1	2	0	0	0
	8	6	4	14	4	10	0
		7	3	10	2	10	10
Potato dextrose..... (dehydrated)	3	6	1	8	6	10	0
		8	0	8	2	0	0
	4	2	0	6	0	10	0
		0	0	4	2	10	10
	8	10	2	22	8	10	0
		6	4	6	4	10	10
Malt.....	3	6	0	14	0	10	0
		6	0	8	0	10	0
	4	0	0	4	0	10	0
		2	0	4	2	0	0
	8	2	0	14	4	10	0
		2	0	10	4	10	10
Peptonized milk.....	3	4	0	10	0	20	0
		6	0	10	0	10	0
	4	6	4	8	0	10	0
		5	0	4	0	20	0
	8	5	0	14	4	20	0
		7	0	6	0	20	0
Whey.....	3	6	3	10	2	10	0
		4	0	8	0	0	0
	4	0	0	2	0	0	0
		0	0	0	0	0	0
	8	10	2	10	0	0	10
		6	3	28	10	20	0
Wort.....	3	1	0	2	0	0	0
		2	0	4	0	0	0
	4	3	6	12	14	20	0
		4	1	10	0	0	0
	8	11	0	18	6	20	0
		16	4	14	2	20	0

sented, however, it is not possible to note any definite tendencies for any one of the culture media used to give consistently higher counts although it should be noted that in practically all instances the mold counts were appreciably lower than the yeast counts. The data ap-

pears to indicate (Table 4) a tendency for higher counts among the duplicate plates of the 1:2 dilutions (5 ml. of the 1:10 dilution) poured with the potato-dextrose agars which apparently was not so consistently exhibited by the other media.

TABLE 4
*Comparative Yeast and Mold Counts of Salted Butter Plated on
Various Culture Media Adjusted to pH 3.5*
(Low-count data)

Media	Sample No.	1 ml. direct		1:2 dilution		1:10 dilution	
		Yeast	Mold	Yeast	Mold	Yeast	Mold
Potato dextrose..... (freshly prepared)	3	16	0	28	0	20	0
		21	0	36	0	30	0
	4	11	3	16	4	30	0
		11	3	20	4	20	10
	8	4	0	10	0	0	0
		4	0	4	0	0	0
Potato dextrose..... (dehydrated)	3	34	0	32	0	30	0
		26	0	36	0	30	0
	4	16	2	18	4	30	0
		17	14	22	4	20	20
	8	4	0	10	0	0	0
		3	2	16	0	0	10
Malt.....	3	14	0	6	0	0	0
		10	0	16	0	20	0
	4	4	16	10	0	20	0
		11	0	24	6	10	10
	8	3	0	4	2	10	0
		0	4	4	4	0	0
Peptonized milk.....	3	9	0	0	4	40	0
		11	0	16	0	10	0
	4	18	0	12	4	10	0
		11	4	6	14	20	10
	8	2	0	10	4	10	0
		3	1	8	2	10	10
Whey.....	3	11	0	16	2	20	10
		24	0	18	0	20	0
	4	11	0	18	0	20	0
		4	6	18	4	20	0
	8	4	0	10	0	10	0
		4	0	4	2	10	10
Wort.....	3	16	0	30	4	0	0
		12	4	16	2	30	0
	4	11	0	12	0	20	0
		12	0	12	0	0	10
	8	4	4	6	0	0	0
		4	3	0	0	0	0

It was noted (Table 3) that there were no definite tendencies for any of the culture media used to give consistently higher counts although it was observed generally that the mold counts were appreciably lower than the yeast counts. Furthermore, greater variation is apparent among mold counts than occurs with the yeast counts. The potato-dextrose agars and the malt agar gave relatively high yeast and relatively low mold counts (Table 5), while the peptonized-milk agar and especially wort agar gave much higher mold counts with their yeast counts tending to be lower than those obtained with the potato-dextrose agars. Apparently the pink yeasts were not fa-

TABLE 5

Comparative Yeast and Mold Counts of Unsalted Butter Having a Definite Mold Infection Plated on Various Culture Media of pH 3.5

(Intermediate-count data)

Media	1 ml. direct		1:2 dilution		1:10 dilution		1:100 dilution	
	Yeast	Mold	Yeast	Mold	Yeast	Mold	Yeast	Mold
Potato dextrose.....	24	2	34	4	30	10	0	0
(freshly prepared)	24	6	30	4	40	10	0	0
Potato dextrose.....	26	4	36	4	40	20	0	
(dehydrated)	22	4	30	4	40	29	0	100
Malt.....	24	0	32	0	40	40	0	0
	24	1	24	2	40	0	0	0
Peptonized milk.....	20	0	28	14	30	10	0	0
	18	3	20	10	30	0	0	0
Whey.....	18	0	26	10	20	10	200	0
	10	0	34	2	20	0	0	0
Wort.....	24	0	26	28	20	10	100	100
	26	4	34	12	40	10	0	0

vored by peptonized-milk, malt, and whey agars as they were by the potato-dextrose agars. These differences were marked in the 1:2 dilutions (5 ml. of a 1:10 dilution), whereas in the 1 ml. direct platings the mold growth appeared to be even more suppressed in the culture media which favored its growth in the 1:2 dilution. The data presented (Table 5) have been confirmed similarly in the examination of another unsalted butter sample wherein the peptonized-milk and wort agars favored the growth of more mold colonies and slightly fewer yeast colonies in the 1:2 dilutions (5 ml. of the 1:10 dilution) than was the case with the potato-dextrose, malt, and whey agars, the total yeast and mold counts ranging from 40 to 50 colonies per ml. It is interesting to note also that the peptonized-milk, whey, and the two potato-dextrose agars gave average total yeast and mold counts

of 36 colonies per ml., while the wort agar gave an average of 50 and the malt agar an average of 29 colonies per ml. Striking similarities were also noted in the case of still another sample of unsalted butter plated on the various media using the 1:2 dilution, indicating that wort agar gave the highest mold counts and in this particular case even gave slightly higher yeast counts than did the potato-dextrose agars.

The data obtained from plating high-count, salted and unsalted butters in the different culture media using the 1 ml. direct plating as well as the 1:2, 1:10, and 1:100 dilutions are shown (Table 6). In the case of salted butter the most consistent counts were obtained in the 1:10 dilutions (1 ml. of the 1:10 dilution) in the potato-dextrose agars and the whey agar, the latter also giving the highest yeast and lowest mold counts. The average total yeast and mold counts of these three media compared favorably. With the unsalted butter reasonably consistent counts were obtained in the 1:10 dilutions (1 ml. of 1:10 dilutions) in all the culture media studied, the lowest total counts being obtained with the peptonized-milk agar and the highest with the dehydrated potato-dextrose, whey, and wort agars which are representative of high-count butters.

It appears that the potato-dextrose agars give the most consistent and highest yeast and mold counts in salted butter; whereas, in the cases of the unsalted butter, the highest and most consistent yeast and mold count was obtained with the wort and whey agars, although the dehydrated potato-dextrose agar had similar tendencies

DISCUSSION

In a study of comparative methods and media for determining the yeast and mold count of butter it is extremely hazardous for one worker to attempt to draw definite conclusions from the results obtained because there are conflicting data and also because the personal equation might unduly prejudice the interpretation of results. Therefore, we are offering no conclusion.

In spite of this decision to be conservative there are some results which permit of definite statements. The data presented show, for example, the necessity of proper acidity adjustment of media in order to eliminate the confusion which the growth of bacterial colonies can introduce. Furthermore, in butter practically devoid of yeast and mold contamination, there apparently was no tendency for appreciable growth of yeast and mold colonies in any of the media studied where the butter sample was plated directly and with the 1:2, 1:10, and 1:100 dilutions employed.

In low-count and intermediate-count butters the use of the 1:2 dilution involving the plating of 5 ml. of a 1:10 dilution invariably gave higher total yeast and mold counts and generally the tendency was for more consistency in the duplicate counts, the main exception

TABLE 6
Comparative Yeast and Mold Counts of Salted and Unsalted Butter Plated on Various Culture Media Adjusted to pH 3.5

(High-count data)

Media	1 ml. direct		1:2 dilution		1:10 dilution		1:100 dilution	
	Yeast	Mold	Yeast	Mold	Yeast	Mold	Yeast	Mold
Salted butter								
Potato dextrose.....	30	10	80	22	140	50	200	100
(ours)	29	11	84	18	130	40	100	0
Potato dextrose.....	30	14	94	30	150	50	200	0
(dehydrated)	26	17	92	18	150	70	100	100
Malt.....	13	1	96	30	140	50	200	0
	19	16	86	14	100	20	100	200
Peptonized.....	19	7	58	14	180	70	200	0
	21	10	70	20	60	80	200	0
Whey.....	36	6	114	6	170	10	100	100
	20	5	120	10	180	30	0	0
Wort.....	16	5	76	20	120	20	0	0
	30	6	60	14	60	20	100	0
Unsalted butter								
Potato dextrose.....	74	10	170	0	180	0	200	0
(ours)	70	3	170	2	160	0	200	200
Potato dextrose.....	70	14	158	10	180	10	200	0
(dehydrated)	70	23	168	4	160	40	1400	0
Malt.....	70	0	134	10	150	0	1200	0
	70	0	156	10	180	10	100	0
Peptonized.....	58	30	115	10	150	10	200	0
	58	10	128	14	140	10	200	0
Whey.....	58	10	78	14	170	10	200	200
	54	18	116	2	180	20	100	0
Wort.....	50	10	180	16	180	30	400	0
	41	30	106	4	180	10	200	0

being low-count, unsalted butters. One very interesting observation was that unsalted butters of intermediate count tended to give higher mold counts with dehydrated peptonized-milk and wort agars, the latter medium apparently tending to give also a maximum yeast

count. Potato-dextrose agar, however, appeared to be equally effective in the case of high-count butters (both salted and unsalted) this being particularly the case with the dehydrated product supplied by the Digestive Ferments Company. Furthermore, in the case of low-count and intermediate-count, salted butters potato-dextrose agar gave more consistent and higher counts than, and compared favorably with, the other culture media studied in the case of low-count, unsalted butters. Thus, with the exception of intermediate-count, unsalted butters, which have an appreciable mold content, potato-dextrose agar—especially the dehydrated products of the Digestive Ferments Company—appeared to have greater utility in determining the yeast and mold counts of butter than did the dehydrated malt, peptonized-milk, whey, and wort agars. This is important to the control laboratory. However, we cannot afford to ignore the exception stated above for wort agar in the case of intermediate-count, unsalted butter having an appreciable mold contamination. In fact the determination of mold colonies in unsalted butter is a subject of considerable speculation to many control laboratories today. Possibly more attention should be devoted to the determination of mold colonies, for in the data presented as a result of this study it is obvious that greater variation occurs in the mold counts than in the yeast counts. There is the possibility, of course, that a pH of 3.5 is optimum for the growth of yeast colonies and is not so favorable to molds and also that other nutrients may be required than those supplied in the media included in this study.

SUMMARY

A study of comparative methods for determining the yeast and mold counts of salted and unsalted butters was made, using as culture media freshly prepared potato-dextrose agar and the dehydrated potato-dextrose, malt, peptonized-milk, whey, and wort agars prepared by the Difco Laboratories.

In the data accumulated as the result of this study the following observations and tendencies were noted:

1. The reaction of the various culture media was adjusted to pH 3.5 in order to avoid the confusion that the growth of bacterial colonies introduced when the reaction exceeded pH 4.7.

2. In butters having a total yeast and mold count not in excess of 30 colonies per ml. the 1:2 dilutions (5 ml. of a 1:10 dilution) tended to give higher counts than 1 ml. of melted butter plated directly irrespective of the media used. In the case of unsalted butters the different media studied did not appear to influence the results materially; whereas, in the case of salted butters more con-

sistent results appeared to be obtained with potato-dextrose agar (either freshly prepared or dehydrated) than with the other media included in this study.

3. In unsalted butters having an intermediate yeast and mold count ranging from 40 to 50 colonies per ml. plating in a 1:2 dilution (5 ml. of 1:10 dilution) on wort agar apparently gave as satisfactory counts of yeast colonies as, but greater growth of mold colonies than, did the other culture media studied.

4. In butter having a total yeast and mold count of approximately 200 colonies per ml. the 1:10 dilution gave the most consistent results. In salted butter the media giving the highest mold counts as well as the most consistent high total counts were the potato-dextrose agars; whereas, in unsalted butter wort agar appeared to be the preferred medium.

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TYPES OF BACTERIA SURVIVING IN FROZEN-PACK VEGETABLES¹

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INTRODUCTION

The effect of freezing on microorganisms naturally occurring in frozen-pack vegetables and fruits has been studied chiefly from two points of view. Quantitative investigations by various authors—Prescott, Bates, and Highlands (1932); Berry (1933); Brown (1933); Smart (1934); Lochhead and Jones (1936); and Smart and Brunstetter (1937)—have indicated the number of microorganisms surviving after different periods of storage at various temperatures of freezing. Such studies have shown that although pronounced decreases in numbers generally occur, particularly during the first few weeks of storage, the decline later becomes less pronounced. Even after months or years in the frozen condition vegetables and fruits may still contain appreciable numbers of organisms capable of developing after defrosting. The process is not one of sterilization.

On the other hand, studies from a qualitative standpoint have been reported by a number of investigators: Brown (1933), Smart (1934), Tchistiakov and Noskova (1936), and Smart and Brunstetter (1937). From frozen-pack vegetables and fruits numerous genera and species of bacteria, yeasts, and fungi have been described; and in some cases, comparisons were made with organisms similarly isolated from freshly packed products. Concerning the effects of freezing on the relative incidence of the various types found and their relative susceptibility to freezing, comparatively little information is available.

From the standpoint of both food quality and public health the qualitative nature of the organisms surviving in frozen-pack products is a matter of great importance. At the same time the relative abundance of different groups of organisms and the differential effect of freezing on them are likewise important considerations. The changing proportion of various types while the freshly packed product lies in storage and the relative abundance of possible food-poisoning types (e.g., *Staphylococcus*, *Salmonella*, etc.) at the time of defrosting may well be more important considerations than the simple presence or absence of certain species.

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Preliminary studies already reported by Lochhead and Jones (1936) have indicated that species of *Micrococcus*² and *Flavobacterium* appear to withstand freezing better than other types of bacteria occurring in fresh-pack vegetables. Organisms of these genera were found to comprise a noticeably greater percentage of those surviving after nine months' freezing at $-17.8^{\circ}\text{C}.$ ($0^{\circ}\text{F}.$). In view of the increasing importance being attributed to staphylococci in matters pertaining to food hygiene, additional studies were made on the quantitative-qualitative aspects of bacteria in frozen-pack vegetables.

EXPERIMENTAL PROCEDURE

The material studied consisted of asparagus, spinach, peas, beans, and corn packed by the Division of Horticulture, Central Experimental Farm. The vegetables were prepared by blanching in boiling water and packed in one-pint containers with sufficient brine, varying from two to three per cent with the product, to cover the solid portions. For comparative purposes two types of containers were used, paraffin-lined cartons and sealed cans. The products were stored promptly in a freezer at $-17.8^{\circ}\text{C}.$ ($0^{\circ}\text{F}.$) and kept frozen until tested.

For analysis duplicate packages of each type were defrosted at $37^{\circ}\text{C}.$ ($98.6^{\circ}\text{F}.$) and tested as soon as the contents were workable. For quantitative tests 100 grams of the solid material were removed aseptically to a sterile jar and 50 ml. of liquid from the package added. The material was mashed thoroughly and 100 ml. of sterile water added. The jar was shaken vigorously 200 times and further dilutions made as required by adding 10-ml. portions to 90-ml. sterile water blanks. Bacterial numbers were estimated on standard nutrient agar, plates being incubated at $4^{\circ}\text{C}.$ ($39.2^{\circ}\text{F}.$) for 21 days, at $20^{\circ}\text{C}.$ ($68^{\circ}\text{F}.$) for 4 days, and at $37^{\circ}\text{C}.$ ($98.6^{\circ}\text{F}.$) for 2 days.

For the qualitative studies four agar plates from the $20^{\circ}\text{C}.$ series were selected, representing two containers of each type for each vegetable studied. From the plates all colonies, or all colonies in even sectors of the plates, were transferred to agar slants. Cultures were compared microscopically and macroscopically and sufficient physiological tests made to permit the elimination of similar types and to establish the generic classification. The frequency of the various types was then estimated.

INCIDENCE OF BACTERIA TYPES IN FRESH AND FROZEN VEGETABLES

The percentage distribution of different groups of organisms in fresh vegetables and in the same products after eight months' storage at $-17.8^{\circ}\text{C}.$ ($0^{\circ}\text{F}.$) is shown (Table 1), based on a study of 2,310

²In this paper the term *Micrococcus* is intended to include *Staphylococcus*.

TABLE 1

Percentage Distribution of Different Bacteria Groups in Fresh and Frozen Vegetables—1955 Pack

	Asparagus		Spinach		Peas		Beans		Corn	
	Fresh pack	Frozen 8 mos.	Fresh pack	Frozen 8 mos.	Fresh pack	Frozen 8 mos.	Fresh pack	Frozen 8 mos.	Fresh pack	Frozen 8 mos.
Number of colonies studied.....	200	134	175	185	296	270	300	300	300	150
Proportion in which no growth occurred on transfer.....	8.5	3.7	Nil	0.5	7.1	3.7	2.7	Nil	Nil	Nil
Proportion in which growth took place.....	91.5	96.3	100.0	99.5	92.9	96.3	97.3	100.0	100.0	100.0
Classification										
<i>Coccaceae</i>										
<i>Micrococcus</i> (incl. <i>Staphylococcus</i>).....	25.5	85.8	8.6	63.2	21.3	44.4	7.0	72.0	20.7	78.7
<i>Spirillaceae</i>										
<i>Vibrio</i>	1.7	1.1	2.2
<i>Bacteriaceae</i>										
<i>Serratia</i>	0.5	0.8	2.9	0.7
<i>Flavobacterium</i>	12.5	3.7	32.0	24.9	14.9	42.2	38.3	11.4	7.7
<i>Chromobacterium</i>	1.0
<i>Pseudomonas</i>	2.9	2.3
<i>Achromobacter</i>	47.0	42.2	5.4	42.9	3.0	14.6	28.0	3.3
<i>Escherichia</i>	1.5
<i>Aerobacter</i>	0.6	10.7
<i>Salmonella</i>	2.5
Gram-positive rods.....	3.0	3.4	0.3	15.3
<i>Bacillaceae</i>										
<i>Bacillus</i>	0.5	1.5	5.7	2.2	13.2	4.5	1.3	11.6
<i>Actinomycetaceae</i>										
<i>Actinomyces</i>	0.3
Yeasts.....	0.5	1.5	2.7	36.7	19.0	18.0

colonies. The results confirm the findings of tests on frozen packs of the previous year as reported by Lochhead and Jones (1936) when similar quantitative-qualitative studies were made with asparagus, peas, beans, and corn, and data secured from 2,887 colonies from plate cultures. Micrococci and species of *Flavobacterium* were again found to survive freezing relatively better than other forms encountered in the fresh pack. Micrococci in particular showed greater relative survival than other forms, comprising a noticeably higher proportion of the total numbers in the frozen than in the fresh pack, as shown by comparative figures for the two years' packs (Table 2).

TABLE 2
Percentage of Micrococci in Fresh-Pack and in Frozen Products

Product	Number of colonies studied	1934 pack		1935 pack	
		Fresh	Frozen 9 mos.	Fresh	Frozen 9 mos.
Asparagus.....	1,123	pct. 0.3	pct. 33.2	pct. 25.5	pct. 85.8
Spinach.....	360	8.6	63.2
Peas.....	1,259	18.5	26.0	21.3	44.4
Beans.....	1,241	5.2	52.9	7.0	72.0
Corn.....	1,214	11.8	37.7	20.7	78.7

Achromobacter species, which as in the previous year's test comprised in most cases a considerable proportion of the bacteria in the fresh pack, declined usually to a marked degree during storage. The proportion of spore-forming bacteria showed no general tendency to increase in the frozen products; indeed in three cases out of five decreases were actually noted. These findings, though agreeing with previous studies by the writers (1936), are at variance with a general belief that the proportion of spore-forming organisms is greatly increased in frozen vegetables. In support of this, however, no data of a quantitative nature have come to our attention.

EFFECT OF FREEZING ON ORGANISMS DEVELOPING AT DIFFERENT TEMPERATURES

To note the effect of freezing on the numbers of bacteria capable of developing at different temperatures, counts were made on the freshly packed vegetables and on the same products frozen for eight months. Parallel sets of plates were incubated at three temperatures: 4°C.(39.2°F.) for 21 days, 20°C.(68°F.) for 4 days, and 37°C.(98.6°F.) for 2 days (Table 3).

In all cases maximum counts were obtained at 20°C., indicating that not only in the freshly packed but also in the frozen product

the majority of organisms are types which grow best at moderate temperatures. Even after eight months' freezing the surviving bacteria may be regarded as cold-resistant rather than cold-loving. The results also show (Table 3) that with increasing temperature of incubation a greater relative survival of the organisms is recorded. The greatest percentage reduction in numbers occurred with bacteria growing at low temperature, while those growing at 37°C. (98.6°F.) showed proportionately the least reduction, a finding which appeared surprising with products held in storage in the frozen state.

In explanation two possibilities suggested themselves—an increase in optimum growth range owing to freezing and a difference in types of bacteria from frozen products appearing at different temperatures. Twenty pure cultures were tested as to growth at eight tem-

TABLE 4
*Effect of Temperature on Micrococci Developing From Frozen Vegetables—
1936 Pack*

Product (frozen four months)	4°C. (39.2°F.)		20°C. (68°F.)		37°C. (98.6°F.)	
	Colonies examined	Micro- cocci	Colonies examined	Micro- cocci	Colonies examined	Micro- cocci
		pct.		pct.		pct.
Asparagus.....	12	8.3	54	11.1	20	70.0
Peas.....	80	2.5	100	58.0	80	70.0
Beans.....	24	0.0	104	23.1	68	38.2
Corn.....	68	0.0	100	36.0	108	66.6

peratures ranging from 2.2°C. (36°F.) to 45°C. (113°F.). They were also inoculated into sterile corn juice, frozen at -17.8°C. (0°F.) for one week, and then re-tested. No changes in growth range or optimum temperature were observed. The explanation was suggested rather by a qualitative study of the types of bacteria developing at the three incubation temperatures. Asparagus, peas, beans, and corn packed the following year (1936) were examined after freezing for four months and the relative abundance of micrococci determined. The results indicate that as the temperature increases micrococci form a larger proportion of colonies on the plates (Table 4). Since micrococci are relatively the most resistant to freezing (Tables 1 and 2), the proportionately greater resistance of organisms at 37°C. may be understood.

The greater relative survival to prolonged freezing shown by micrococci as compared with other bacteria occurring in freshly packed vegetables appears to be a matter of more than academic interest. In recent years increased attention has been focused on this group of organisms as active agents in outbreaks of food poisoning.

This has been especially noted in cases involving gastro-enteric disturbances following the ingestion of certain starchy foods from which micrococci capable of producing enterotoxin have been isolated. The survival of micrococci in frozen vegetables, their relative incidence, their capacity for development at different temperatures in defrosted products, and their toxin-producing properties are all questions of importance in the development of the frozen-pack industry. Further studies now under way include an investigation of the types of micrococci surviving in frozen vegetables and their enterotoxic properties.

SUMMARY

In confirmation of previous studies, micrococci and species of *Flavobacterium* were found to be relatively more resistant to freezing than other types of bacteria encountered in frozen vegetables. Micrococci in particular comprised a much larger percentage of the organisms in the frozen products as compared with the freshly packed products. Data are based on results from the examination of a total of 5,197 colonies.

There was no definite evidence that spore-forming rods were proportionately more numerous in frozen than in freshly packed vegetables.

Comparative tests of organisms growing at 4°C.(39.2°F.), 20°C.(68°F.), and 37°C.(98.6°F.) showed that 20°C. permitted development of the greatest numbers, both in freshly packed vegetables and in the same products frozen for eight months at -17.8°C.(0°F.). It was found, however, that organisms developing at 37°C. were proportionately the most resistant, and those developing at 4°C. the least resistant to freezing. The explanation is believed to lie in the fact that as the temperature of incubation is increased micrococci form a higher proportion of the organisms developing.

The findings direct attention to the possible importance of the *Micrococcus* group of organisms in relation to the proper handling of frozen vegetables.

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CHANGES IN IRON CONTENT OF MUSTS AND WINES DURING VINIFICATION

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There is very little available information concerning the changes in iron content of California musts and wines occurring during vinification. Byrne, Saywell, and Cruess (1937) found that the iron content of wines made in glass containers decreased during fermentation. Mrak, Cash, and Caudron (1937) discussed briefly the literature concerning the effect of metals on wines.

In the present investigation several series of samples were obtained from four wineries equipped with iron crushers and must lines. These were located in Sonoma (G and H), Napa (N), and Santa Clara (SC) counties. Samples from the same lot of grapes were obtained in the winery at several stages during vinification. It was not always possible to obtain a complete series of samples because of blending and variations in the vinification procedure. All samples, except the three in Series H 1, were obtained after the equipment had been in use for several hours. The samples in Series H 1 were taken early in the morning at the start of operations for that day. All samples collected were placed in glass-top jars and preserved with formaldehyde until analyzed. Iron determinations were made by the method of Stugart (1931).

Results of analyses show that the iron content of juice from the musts increased during crushing and passage through the must lines but decreased greatly during fermentation (Table 1). The iron may have been absorbed by yeasts or precipitated with the lees in some other manner. The variations in iron content of the musts and wines of the different series may have been caused by any of several factors. One very important factor was the time at which the sample was taken. Series H 1 was taken early in the morning and was representative of the first grapes or musts passing through the crusher and must line. Although the crusher and must line were thoroughly washed at the conclusion of each day's operation, some juice probably remained on the equipment and, in presence of large amounts of air, corroded it. In the morning the first lot of grapes that passed through the crusher and must line dissolved the iron salts and thus picked up sufficient iron to reach an iron concentration of 430 parts per million.

TABLE 1
Changes in Concentration of Iron in Musts and Wines During Manufacture
(Given in parts per million)

Source of sample		Red juice and wine series								White juice and wine series				
Series No. ¹	H 1	SC 1	SC 2	SC 4	SC 5	SC 6	SC 7	SC 8	N 2	H 1	G 1	SC 3	H 2	N 1
Fresh grapes (juice).....	6.5	14.2	25.5	8.5	8.5	8.0	11.5	11.0	9.5	4.3	7.8	8.5	2.3	6.3
Crusher (juice from crushed grapes).....	27.8	35.5	9.5	24.8	8.7	14.3	16.0	52.8	12.8
End of must line (juice).....	11.3	38.8	83.3	29.8	26.3	25.5	32.5	15.5	33.0	430	14.7	11.0	10.5
Fermenter (during fermentation).....	45.8	33.5	4.0	7.8	16.3	7.0	10.3	2.0
Fermenter (end of fermentation).....	13.5	11.0	10.3	2.0	5.5	5.5	2.8	3.2
Sump (wine from fermenter).....	15.3	8.3	8.7	4.5
Storage (wine 1 to 3 months old).....	4.8	15.5	8.7	11.3	5.0
Lees from bottom of fermenter.....	53.0
Lees from bottom of storage tank.....	9.0

¹ G and H designate samples from Sonoma County; N, from Napa County; SC, from Santa Clara County.

This particular series was not followed to completion because the must containing the high concentration of iron was mixed in the fermentation vat with other musts containing much less iron.

The increase in iron content in most of the samples of the red wine series was much greater than in the white wine series. The actual amount of iron lost by red musts during fermentation was greater than that lost by the white musts, but when the losses were expressed as percentages they were approximately within the same range.

Three of the five samples obtained from storage tanks contained concentrations of iron slightly higher than did the samples obtained from the fermenters. These increases were probably caused by contact with iron during the handling of the wines.

The single sample of lees¹ (yeast sediment) obtained from a fermentation tank contained a relatively high concentration of iron, whereas the sample of lees obtained from a storage tank had a relatively low concentration.

Since much of the iron dissolved by must is removed during fermentation it appears that iron contamination by contact of must with iron equipment is less serious than the iron picked up by the wine during cellar treatment.

SUMMARY AND CONCLUSIONS

The iron content of several series of samples of musts and wines obtained at various stages of commercial wine making was determined.

Considerable quantities of iron were absorbed by musts during passage through iron crushers and must lines.

Most of the iron present in the juice (must) was precipitated out of it during fermentation.

NOTE

Since this paper was submitted for publication, Schanderl (1938) has reported the absorption of iron by yeast from fermenting must.

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¹Crude lees consist mainly of yeast and other organic matter, whereas wine lees consist largely of argyls.

VITAMIN C CONTENT OF VEGETABLES

IX. INFLUENCE OF METHOD OF COOKING ON VITAMIN C CONTENT OF CABBAGE¹

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INTRODUCTION

The vitamin C content of cabbage has been extensively studied because of the high antiscorbutic potency of this vegetable. The amounts of ascorbic acid reported in raw cabbage have differed greatly. Clow and coworkers (1929) observed only .1 to .2 mg. per gm.,² while Birch, Harris, and Ray (1933) found .9 to 1.0 mg. per gm. Probably because of the great variety of methods used, the amounts of vitamin C reported in cooked cabbage have varied still more. Holst and Frölich (1912) reported a great loss in the antiscorbutic potency of cabbage during cooking. Their results have been confirmed by a number of later workers—Delf (1918), Eddy and coworkers (1923), and Scheunert (1930)—but more recently much smaller losses have been observed. Bukin, Izmailova, and Bogochunaz (1934) reported a loss of 50 per cent after one hour's cooking; Jarusova (1935), a destruction of 20 per cent; Vinokurov, Eidelman, and Butom (1935), a destruction of 1 to 10 per cent; Halliday and Noble (1936), no destruction after cooking 10 minutes but a loss of 69 per cent caused by vitamin C dissolving in the cooking water. Although Ahmad (1935), Guha and Pal (1936), and Levy (1936) reported an increase after boiling cabbage, Mack (1936) has shown that the greater part of this apparent increase is due to the inactivation by heat of the so-called oxidizing enzymes.

Because of the great differences in ascorbic acid losses reported in cooked cabbage, it was thought that a comparison of three methods of cooking (boiling, steaming, and panning) with especial regard to the total destruction of the ascorbic acid and its extraction by the cooking water might be of value. In boiling and steaming the effect of cutting the cabbage into different sized pieces was observed. The

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² Recalculated on the basis of the requirement of .5 mg. of ascorbic acid per day for the protection of a 300-gram guinea pig from scurvy, according to Waugh and King (1932).

following experiments were all performed with the same variety of cabbage freshly harvested at the same stage of maturity.

A check on the chemical method by biological assay was not considered necessary because of the close agreement obtained with these two methods by Bessey and King (1933) and by Gould, Tressler, and King (1936).

EXPERIMENTAL PROCEDURE

The variety of cabbage used in these experiments was the Glory of Enkuizen. It was grown on Ontario clay loam near Geneva, New York, and harvested October 20, 1936, at optimum maturity. The method of analysis was that of Bessey and King (1933) as modified by Mack and Tressler (1937). The acid used for extraction was 1N sulfuric acid containing two per cent metaphosphoric acid.

The method of boiling the cabbage followed essentially that proposed by Halliday and Noble (1933) and used by Gould, Tressler, and King (1936). Portions of cabbage, each weighing 360 grams, were finely shredded, cut in strips one-half inch wide, and in quarters and dropped into 1,800 ml. of briskly boiling water. After two minutes, required for the water to come back to boiling, the finely shredded cabbage was cooked 8 minutes; the strips, 10 minutes; and the quarters, 16 minutes. By a manometer device the flow of gas was regulated for each cooking to give a large amount of heat for the first two minutes, followed by a smaller amount sufficient to produce fairly rapid boiling and maintain a uniform rate of evaporation.

At the end of the boiling periods the cooking water was drained from the cooked cabbage for one minute through a strainer. Both cabbage and water were weighed. Duplicate samples of boiled cabbage and cooking water were placed in 25 ml. of chilled acid and analyzed. The moisture content of the cooked and raw cabbage was also determined.

In the steaming procedure the same quantities (360 gm.) of cabbage, finely shredded and in quarters, were put in a steamer after the water had begun boiling briskly. The upper half of the enameled steamer used was eight and one-half inches in diameter at the top, six inches at the bottom, and two and one-half inches deep. Steam entered through the holes around the top rim. Part of this escaped under the cover; part of it condensed and dripped back over the cabbage, leaving, together with juice from the cabbage, approximately 35 ml. of water with the finely shredded cabbage and 50 ml. with the quartered cabbage. To reach the cooked stage the finely shredded cabbage required 14 minutes of steaming and the quartered, 25 minutes of steaming. The gas pressure was regulated to give the same amount of evaporation throughout.

After being weighed, the steamed cabbage and the remaining juice were analyzed for ascorbic acid and moisture.

In panning, 360 grams of finely shredded cabbage with one teaspoon of Crisco and three-fourths teaspoon of salt were heated very slowly for 10 minutes until enough juice for cooking had been drawn out of the cabbage. After this the heat was gradually increased. The cabbage was cooked an additional 10 minutes to the "done" stage. Because of the necessity of drawing out as much juice as possible in a short time, finely shredded cabbage only was used for panning.

DISCUSSION

From a comparison of the methods of boiling cabbage, it is shown that finely shredded boiled cabbage with the cooking water retained a greater amount of ascorbic acid than if it were boiled in strips or quarters; however, the extraction of ascorbic acid by the cooking water was greatest in this case, probably on account of the larger cut surface.

From data obtained by Gould, Tressler, and King (1936), showing that the greatest destruction of vitamin C occurs before the cooking water begins to boil and that very little is lost thereafter, it is apparent that the greater destruction occurring when cabbage was cooked in strips or quarters was not caused by the longer period of cooking. The fact that heat does not penetrate the larger pieces as quickly as it does the smaller pieces and thus more time is required for the inactivation of the so-called oxidizing enzymes, may explain the greater total destruction occurring in the strips and quarters.

If used for soup, cooking water from finely cut cabbage would result in a more nutritive product than the cooking water from the strips and quarters. On the other hand if the cooking water is to be discarded, it is advantageous to boil the cabbage in strips.

In the case of steamed cabbage, the large surface of the finely shredded pieces exposed to atmospheric oxidation probably accounted for a total destruction of 26 per cent, which was greater than for the boiled cabbage of the same cut. This is in accord with the theory of Holst and Frölich, that the destruction of ascorbic acid by cooking is less when air is excluded during heating. The smaller amount of ascorbic acid destroyed in the steamed, quartered cabbage can be explained by the same theory. The high percentage of ascorbic acid dissolved from the finely shredded, steamed cabbage probably resulted from the steam condensing and dripping over the large cut surface. For this reason the finely shredded, steamed cabbage did not retain much more vitamin C than drained, finely shredded, boiled cabbage. The percentage of ascorbic acid, however, retained by drained, quar-

TABLE 1
Ascorbic Acid Content of Glory Cabbage Before and After Cooking by Various Methods¹

	Number of experi- ments	Before cooking— wet wt. basis	After cooking— wet wt. basis	Cooking water	Original ascorbic acid		
					In drained cabbage	In cooking water	Destroyed
		mg. per gm.	mg. per gm.	mg. per gm.	pct.	pct.	pct.
Boiled cabbage							
Finely shredded.....	4	.36±.014	.09±.006	.050±.004	22±.6	66±.5	12±.6
Strips.....	3	.34±.018	.13±.006	.036±.003	32±1.3	53±1.1	15±1.5
Quarters.....	4	.37±.020	.13±.006	.045±.004	38±1.1	56±1.5	16±2.5
Steamed cabbage							
Finely shredded.....	4	.33±.005	.23±.004	.223±.006	24±.1	50±.4	26±.4
Quarters.....	4	.35±.011	.26±.006	.185±.007	58±1.1	22±.3	10±1.0
Panned cabbage							
Finely shredded.....	4	.27±.008	.20±.007	None	66±1.3	None	31±.8

¹ Showing the probable error.

tered, steamed cabbage was more than double the amount retained by drained, finely shredded, steamed cabbage.

In panned cabbage the 10 minutes' heating in which the large cut surface was exposed in the absence of an appreciable quantity of steam, evidently caused a great deal of irreversible oxidation, which resulted in a greater total destruction of vitamin C than with the two other methods of cooking. But since there was no excess cooking liquid, and none of the ascorbic acid was extracted by the cooking water, the cabbage itself retained the greatest amount of vitamin C when cooked by this method.

In the case of boiled cabbage there was a considerable increase in moisture content; in steamed cabbage, a very slight increase; and in panned cabbage, a considerable loss.

SUMMARY

As determined by the 2,6 dichlorophenol indophenol method raw Glory cabbage was found to contain about .32 mg. of ascorbic acid per gm., and therefore can be considered a good source of vitamin C. Boiling destroyed less than one-sixth of the original ascorbic acid. However, when finely shredded cabbage was used, about two-thirds of the ascorbic acid was extracted by the cooking water. When larger pieces of cabbage were boiled, the amount extracted was less.

Steamed cabbage contained more ascorbic acid than drained, boiled cabbage. Although the amount of ascorbic acid destroyed was larger than in boiling, very little was extracted by condensing steam.

About one-third of the original ascorbic acid was destroyed in panning cabbage. Since no additional water was used for cooking, there was no loss by extraction. Therefore, the panned cabbage itself contained about two-thirds of its original ascorbic acid content.

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LACTIC ACID-PRODUCING BACTERIA IN FERMENTATIONS AND FOOD SPOILAGE¹

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In a comparative study of fermentations, whether desirable or undesirable, one is immediately confronted with the question of the type of organism responsible for the change. If the change is desirable, a knowledge of the type of organism frequently helps where it is desired to duplicate the fermentation. If an undesirable effect is produced, a knowledge of the organism may be of value as a means of eliminating the organism. Wherever a natural fermentation of plant material is noted in which there is a reduced oxygen tension, it can be assumed that a lactic acid fermentation is being carried out by a gram-positive rod or coccus. It is difficult to say where these organisms originate, but they are ever present when opportunity is given for growth. Because of the variety of materials from which they have been isolated, many persons have a badly confused idea of the nature of these lactic acid bacteria. If more attention had been given to the type of change which takes place in fermenting materials and less to the material itself, such confusion as there is at present would not exist.

Gram-positive, non-motile, lactic acid-producing bacteria have been isolated from a great variety of sources. In studying these organisms, actually only a few really distinct types have been found. The group is important not only because of the undesirable changes they bring about in food products, but also because of the many desirable changes they bring about in other products. The purpose of this paper is to note some of the similarities in changes in what are apparently divergent types of fermentation.

The gram-positive, non-motile, lactic acid bacteria resemble each other in many characteristics. They may be classified either on the basis of morphology or on the basis of the type of fermentation produced. By the morphological classifications we have the cocci of the genera *Streptococcus* and *Leuconostoc* and the rods of the genus *Lactobacillus*. By the physiological grouping the non-gas-producing lactobacilli (*Thermobacterium* and *Streptobacterium* of Orla-Jensen) are

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allied with the streptococci, while the gas-producing lactobacilli (*Beta-bacterium* of Orla-Jensen) are grouped with the gas-producing cocci or *Leuconostoc*. This physiological grouping is the more important one for the purposes of the present discussion.

The non-gas-producing organisms—either rods or cocci—are those which produce from 85 to 90 per cent or more of lactic acid—inactive, laevo, or dextro—from the hexose sugars with only slight amounts of other by-products, such as carbon dioxide and acetic acid. When pentose sugars are fermented these are ordinarily converted into equal molecular quantities of lactic and acetic acid. The group as a whole has very little action on protein but is rather fastidious in regard to the source of nitrogen utilized in growth. It is this fact which leads to difficulty in the isolation of pure cultures. Apparently none of the group has the power to split proteins in order to secure their nitrogen but must obtain it from free groups of protein molecules.

The gas-producing types also find it difficult to grow, primarily because of the necessity of using a proper nitrogen source for growth. When the aldohexose sugars are fermented, approximately one-half of the sugar is converted to lactic acid, one-fifth to carbon dioxide, and the remainder to acetic acid and ethyl alcohol. The lactic acid produced by the cocci is apparently always of the laevo form while that produced by the rods is always of the inactive form. These are the normal fermentation by-products but certain conditions may alter the products slightly. Only acetic and lactic acid are produced from the pentose sugars. One of the major end products from fructose is mannitol. The amount which can be recovered is dependent upon the availability of this substance to the particular strain or strains causing the fermentation. In addition to these products many of the coccus forms have the peculiar characteristic of producing a slimy growth owing to dextran production when sucrose is the sugar fermented. This variety of end products from fermentation has frequently caused confusion in the literature.

Strains of the genus *Leuconostoc* were described as early as 1878 as the bacteria that causes the slime development, or "frog spawn fungus," in sugar factories, particularly when growing in the raw juices. At times this slime development is so serious that the pipes through which the raw juices are pumped are entirely clogged and it is necessary to stop operations until they can be cleaned out. Usually, unless other measures are taken to rid the equipment of the organisms, the condition reappears. It is surprising that an organism which causes such a serious difficulty in sugar factories should be identical with the one which causes the early fermentation of sauer-

kraut; and furthermore, that this early fermentation is absolutely essential to a good fermentation, a clean flavored product, and the production of carbon dioxide. To a large extent this fermentation is based on the desirable anaerobic condition so produced.

The "frog spawn fungus" has also been found to be the organism which causes the swelling of canned tomatoes, canned fruits, and similar food products. In fact, the majority of swelled cans of tomatoes examined by the author were spoiled by strains of *Leuconostoc mesenteroides*. It is true that the swelled cans of tomatoes were not marketable, but in similar fermentations a truly edible product has been produced.

This same organism is allowed to grow in pineapple juices in order to produce a characteristic slightly acid and alcoholic slimy drink much liked by some people.

A few years ago a small company in western New York manufactured a palatable drink from tomato juice by fermentation with these particular organisms. Possibly they would still be in operation if they could have controlled the yeast fermentation sufficiently so as to keep the alcohol development down to the limits fixed by the prohibition laws then in force. Palatable catsup has been prepared from tomato juice after it has been fermented with strains of *Leuconostoc* to produce the desired lactic acid acidity. This process is not regarded as desirable, however, because the bright red color of the product is destroyed in the fermentation process. A great amount of the catsup of the period previous to about 1915 was made from pulp which had been fermented by these organisms. The organism is one of the general group causing mannitic fermentation of wine, a spoilage which occurs particularly in Europe and was studied years ago. It is not as important as other types of wine spoilage which will be mentioned later.

A very closely related type, *Leuconostoc citrovorus*, has been isolated and described as the organism causing the desirable aroma in butter starters.

The gas-producing strains of the genus *Lactobacillus*, so similar physiologically to the *Leuconostoc*, are equally important. They are the true mannitic bacteria of "sick" wines. Similar types have been in beers and distillery yeast but not recognized as mannite-forming organisms. This is natural since mannite, a reduction product of fructose, is not produced in such products. While gas-producing strains of lactobacilli have always been considered harmful to the wines, it is now a question whether they may have some beneficial value in fermentations of grape juices if properly controlled.

It is also quite apparent that there really are two groups of mannite-forming bacteria: one related to the organism isolated from compressed yeast, the other to the organism found in milk products. Although the organisms are different and intermediate types may be noted, they are frequently found in the same sort of materials. In wine, beer, compressed or distillery yeast, soured bread dough, sour mash, molasses, canned tomatoes, catsup, canned peas, corn, or even sauerkraut they cause undesired fermentations and are therefore considered true spoilage organisms. They are essential in the fermentation of sauerkraut, however, not only because of the high acidity but also because of the carbon dioxide gas that they produce. They are also important in cheese ripening and in silage fermentations. They can, in fact, be obtained from any fermenting plant material in which partial anaerobiosis is brought about.

Since some of these types are so active in their fermentation of pentose sugars, they have been considered for the active fermentation of the pentose sugars in the commercial production of lactic acid and acetic acid.

A smaller group of the mannite formers is even more fastidious in its growth requirements. Undoubtedly the members of this group are entirely overlooked in most fermentations because they not only require certain definite proteins as their source of nitrogen but they also fail to grow, or grow but poorly, with dextrose as the source of energy. They rather require fructose or the pentose arabinose. Such organisms have been obtained from kefir grains, "sick" wine, fermented salad dressing, and beer. Coming from such widely different materials and being so difficult to culture, it is not easy to distinguish one of them from another.

The non-gas-producing, lactic acid rods are a better-known group, particularly the species *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. These, or closely related types, have been found not only in milk drinks and cheese products but also in the alimentary canals of normal individuals as well as of those suffering from gastric carcinomas or carious teeth. In general they are not only high acid-producing types but they prefer somewhat higher temperatures than the other gram-positive, lactic acid bacteria. Similar types, that ordinarily will not grow in milk, have been obtained from soured mash made from various plant materials, and they have been used as the fermenting organism in the commercial production of lactic acid from molasses or other similar material.

Far more common types of non-gas-producing, lactic acid rods are those which grow at lower temperatures. They are commercially

important in a number of fermentations. They are essential organisms in ordinary cheese ripening, in cucumber-pickle fermentation, in sauerkraut in which they are responsible for high acid production, and in silage fermentations. Although various strains isolated from such materials have shown a different fermentation as well as other characters, the same differences found may be obtained between a series of organisms from only one of the sources. Identical organisms are also found as the causative organisms in spoilage of various tomato products, beers, mashes, and occasionally in canned vegetables.

The presence of any of the gram-positive, lactic acid-producing bacteria need not be looked upon with alarm since the changes they bring about in the food product are not detrimental to health. Of the hundreds of cases of illness caused by consumption of food, few have ever been attributed to food in which changes were brought about by these organisms. These few cases have been doubtful. The only effect they possibly may have is in producing acid from sugar to result in an acid food not pleasing to the appetite. In the case of canned foods, the cans nearly always swell and therefore the product never reaches the market.

SUMMARY

The gram-positive, lactic acid-producing bacteria, both rod and coccus forms, may be divided into two physiological groupings: (a) the non-gas-producing types which produce primarily lactic acid in the fermentation of sugars and (b) the gas-producing types which produce alcohol, carbon dioxide, acetic acid, and lactic acid in fermentation of aldohexose sugar; only acetic and lactic acid from pentose sugars; and mannitol from levulose. The cocci also often produce a dextran from sucrose.

These organisms have been isolated from a large variety of sources, not only in desired commercial fermentation but also in many undesired fermentations in which they are definitely considered as spoilage types.

There are relatively few species of lactic acid bacteria. Minor differences between strains are common but not significant. The same differences in a series of strains from various sources may be noted between strains from a single source.

Confusion in the literature has resulted from attaching too much significance to minor differences between strains and from the differences in end products produced from the various energy sources.

HEAT-RESISTANCE STUDIES ON SPORES OF PUTREFACTIVE ANAEROBES IN RELATION TO DETERMINATION OF SAFE PROCESSES FOR CANNED FOODS

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INTRODUCTION

In the determination of adequate sterilizing processes for canned foods capable of supporting the growth of *Clostridium botulinum*, a knowledge of the maximum resistance to heat of spores of *Cl. botulinum* in the products under consideration is of major importance. The maximum heat resistance of spores of this organism in standard phosphate solution at different temperatures has been fairly well established by Esty and Meyer (1922). Unfortunately it is difficult to produce spore crops of strains of *Cl. botulinum* having such a high resistance to heat, even a medium resistance being difficult of attainment. As a result considerable effort was made to find another organism similar in its growth requirements to *Cl. botulinum*, and at the same time having the ability more readily to produce spores of comparatively high heat resistance. A putrefactive anaerobe, known as No. 3679, was isolated from spoiled canned corn by Cameron (1927). It has since been widely used by laboratories connected with the canning industry in experiments on canned foods. McClung's work (1937) indicates definitely that it is not of the parobotulinum species and that it is a different and possibly undescribed species.

Sufficient thermal death-time data have now been obtained to permit a comparison of the heat-resistance characteristics of spores of this organism with those of *Cl. botulinum*. Also the data obtained on *Cl. botulinum* in specific food products are herewith presented and the thermal death-time curve characteristics determined.

These thermal death-time curves closely approximate straight lines on semi-log paper. Ball (1928) developed a method of calculating processes for sealed containers of food using these approximate curves. In his nomenclature the two factors which mathematically describe these curves are given symbols (Fig. 11) and defined as follows:

F—The number of minutes required to destroy the organism (in any specific medium) at 121.1°C. (250°F.).

z—The slope of the thermal death-time curve expressed as degrees F. (This is the interval in temperature required for the line to pass through one log cycle on semi-log paper.)

These two factors, being a point and a slope, determine the thermal death-time curve precisely. The value of *F* is the thermal death time at 250°F. and, when the *z* values are identical, is used to compare the resistance of organisms. If the *z* values are not identical, however, this comparison is invalid, since the ratio of the resistance will vary throughout the temperature range. The *z* value measures the change in thermal death time with changing temperature. As the *z* value diminishes, the effect of temperature change becomes more marked.

This can be seen in the following processes calculated for the same can of food, comparing the *z* values of 14 and 22. The value of *F* is taken as five minutes (250°F.) in both cases:

PROCESS TIME

	Retort temperature 110°C. (230°F.)	Retort temperature 121.1°C. (250°F.)
<i>z</i>		
14	138 minutes	5.11 minutes
22	44 minutes	5.47 minutes

The can temperature at the start of the process was 65.6°C. (150°F.). The heat-penetration factors are $f_h = 3$ and $j = 1.20$, according to Ball (1928).

Owing to variations in heat resistance of suspensions of spores and influence of the medium upon this resistance, the practice was adopted of heating them in a standard phosphate solution (M/15 Na_2HPO_4 and M/15 KH_2PO_4 mixed to give a pH of 7.0) as well as in the particular food under consideration. This permits a comparison of suspensions on a common basis.

If the *z* values are identical the ratio

Resistance in food

Resistance in standard phosphate

may be used. This is known as the food phosphate factor and, while useful, is justifiable only when the *z* values coincide.

The investigation reported in this paper was undertaken to determine: A. Direct thermal death-time data on *Cl. botulinum* in various foods. B. A comparison of the thermal death-time characteristics of Cameron's organism, No. 3679, with *Cl. botulinum*.

The following organisms were selected: (1) No. 3679, (2) *Cl. botulinum*, Type A, No. 62, and (3) *Cl. botulinum*, Type B, No. 213. The culture of No. 3679 was obtained from the Washington labora-

tory of the National Canners Association and the two strains of *Cl. botulinum* from Dr. K. F. Meyer's collection at the Hooper Foundation for Medical Research, University of California.

The spore suspensions of *Cl. botulinum* were grown in a casein-digest medium and concentrated by centrifuging. No. 3679 was grown in peptic digest of liver, veal-gelatin medium and concentrated in the same manner. All suspensions were heated for 10 minutes in boiling water to destroy vegetative cells and stored at 4.4°C. (40°F.) in a small volume of the medium in which they had grown.

EXPERIMENTAL WORK

Containers: Part of the work was done with Pyrex tubes of six to seven mm. inside diameter and one mm. walls, according to the method of Bigelow and Esty (1920), using two c.c. of suspension per tube. The tubes were sealed after filling, and in nearly all cases were subcultured in a medium of glucose, peptic digest of liver, and beef heart after heating. All phosphate suspensions were subcultured after heating.

In most of the experiments on corn and evaporated milk a special can two and one-half inches by three-eighths inch was used. According to the nomenclature usually employed in the canning industry the size would be given as 208 x 006. These are outside dimensions, the depth being taken from the top of the finished double seam to the bottom of the can. This container was designed in the research laboratory of the American Can Company at Maywood, Illinois, especially for thermal death-time work with raw products. The maximum capacity is about 16 c.c., but not more than 13 c.c. of any food material can be safely put into these cans without some overflow in closing. The cans are sealed in vacuum—from 15 to 22 inches of mercury—and incubated without subculturing. Growth usually is determined by bulging of the ends of a can owing to gas formation.

Methods of Heating: Containers heated at 100°C. (212°F.) were suspended in boiling water, and the starting time was recorded from the time the water again came to the boil. This took from one to three minutes. For the other temperatures a battery of seven small retorts was used, operating from the same steam line. Steam was drawn from a large retort automatically maintained at the temperature desired. This apparatus was designed by Richardson (1926) and is shown in the accompanying illustration. This illustration shows part of the large retort, of the vertical three-crate type, with the automatic temperature recorder and controller, the insulated steam pipe from large to small retorts, and two of the seven small retorts. One small retort

at each end of the line is equipped with a mercury thermometer. All have one exhaust valve at the top and three valves at the bottom for drainage, steam, and cold water, respectively (from left to right). Water of condensation in the steam line is taken care of by a trap, and a constant flow of fresh steam is assured by a one-eighth inch bleeder valve at the end of the line. For the containers heated in the small retorts a 20-second period was allowed after the steam was turned on before the start of the process was recorded.

At the start of the heating period the temperature of the containers rapidly rises to that of the bath. Though this lag is not great, it is important when the heating times are short. Richardson (1926)



Retort equipment for determining thermal death times

first investigated this problem; his measurements indicated that the correction for this lag was less than 30 seconds. In view of his results an allowance of 20 seconds was made in the heating times. Recently, however, C. O. Ball, C. C. Williams, and C. M. Merrill studied, in the laboratory of the National Canners Association, Washington, D. C., this question of heat penetration in Pyrex tubes in considerable detail. Based on their unpublished results, the data were corrected as follows: an additional reduction of .4 minute was made in the heating times for phosphate and food juices, and .6 minute for milk. These reductions correct for the lag both in heating and in cooling.

Similar data on the thermal death-time can are lacking, but preliminary work indicates that the correction is less than for tubes; therefore, the original correction of .33 minute was used. This is

probably not great enough, and the true figure lies between this and .73 minute. Thermal death times longer than 15 minutes are not greatly affected by this correction; however, all times less than 40 minutes were corrected in the tables.

At the end of a heating period each retort was filled with cold water in less than five seconds.

Preparation of Foods and Food Juices: Brine from a number of cans of peas, all-green asparagus, and spinach was collected and each lot thoroughly mixed. The juices were then re-canned in cans two and eleven-sixteenths by four inches (211 x 400) and sterilized. Several lots prepared in this manner were required.

The experiments on corn were made using both raw corn and vacuum-packed, whole-grain corn in 208 x 006 cans. In one run only (H5) was cream-style corn used, and this was heated in tubes. One experiment was made to determine the effect on the thermal death time of raw corn as compared with processed corn. Sufficient raw corn was cut for both tests, part being used directly in 208 x 006 cans (Run H19) and the balance canned in 301 x 411 cans and processed for 50 minutes at 115.6°C. (240°F.). This processed corn was used for a thermal death-time run (H20) the following day.

The whole milk, skim milk, and cream were obtained from a factory about 90 miles from San Francisco. They were evaporated and homogenized at the plant, cooled, and brought to the city packed in ice. They were then taken to a local dairy, where the whole milk and cream were heated to 63°C. (145.4°F.) and again homogenized at 3,000 pounds pressure. The samples were cooled and kept at 2 to 4°C. (35.6 to 39.2°F.) until used. In one run (H12) small amounts of sodium bicarbonate and lactic acid were added to determine their effect on the thermal death time. Another variation in the same run was the inclusion of a lot of whole milk of a considerably higher degree of concentration. All runs with No. 3679 were made in 208 x 006 cans, and with the two strains of *Cl. botulinum* they were made in tubes.

Inoculation: For the standard phosphate solution, food juices, milk, and cream, direct inoculations were made from the original spore suspensions, with the exception of the first spore crops of *Cl. botulinum* 213B and 62A. With these, sufficient was taken to give the desired spore concentration in the medium in which they were to be heated, and these amounts were centrifuged. The liquid was poured off, the spores were re-suspended in the food juice or phosphate, then thoroughly shaken with glass beads to break up clumps.

For inoculating the corn in 208 x 006 cans the spores were added in the brine, the concentration being sufficient to give the desired number per container.

The numbers of spores heated in the various runs were as follows:

Suspension	Run No.	Spores per container
No. 3679—Susp. 3	F 8 to F 14, incl.	1,200,000
No. 3679—Susp. 5	G 5 to H 6, incl.	8,000
No. 3679—Susp. 6	H 8 to H 12, incl.	2,000
<i>Cl. bot.</i> 62A, Susp. 1	G 8	10,000,000
<i>Cl. bot.</i> 62A, Susp. 1	H 4	20,000,000
<i>Cl. bot.</i> 62A, Susp. 2	H 10 to H 20, incl.	75,000,000
<i>Cl. bot.</i> 213B, Susp. 1	G 6	12,000,000
<i>Cl. bot.</i> 213B, Susp. 1	H 4	200,000,000
<i>Cl. bot.</i> 213B, Susp. 2	H 10 to H 20, incl.	25,000,000

Filling, Closing, and Subculturing of Containers: All tubes received two-c.c. amounts of the phosphate or food juice to be heated and were then sealed. For subculturing the tubes were scratched with an emery wheel and broken in the flame of a Bunsen burner. The contents were poured into tubes of previously exhausted peptic digest of liver-beef-heart-glucose medium and stratified with sterile vaseline to maintain anaerobic conditions.

The 208 x 006 cans were filled as follows:

Corn.—In runs with No. 3679 each can received 10 grams of corn and 1 c.c. of 8.25-per cent NaCl brine. In runs with the two strains of *Cl. botulinum*, 11.5 grams of corn and 1.1 c.c. of 8.25-per cent NaCl brine were added to each can. The cans were closed in a vacuum of from 18 to 22 inches.

Milk and Cream.—Cans received 11.5 c.c. each and were closed in a 15-inch vacuum. Cultures and cans were incubated at 37°C. (98.6°F.), with the exception of one lot of cans of cream in Run H 12, which was incubated at 24°C. (75.2°F.). This exception was to check against the separation of butterfat at the higher temperature.

Number of Containers.—These containers, tubes or cans, were removed at the end of each time interval.

Incubation.—All cans and subcultures in tubes were incubated at 37°C. for at least three months.

Run Numbers.—Each run is coded with a letter and a number. The letters represent the years and are in sequence, F being 1931. The runs in each year are numbered consecutively, starting with one.

Presentation of Data: The data are presented in two ways: the survival and destruction time data are given for the various media and suspensions (Tables 1 to 5) and the factors of the various ther-

TABLE 1
Survival and Destruction Times for Organism No. 3679 Subcultured in Tubes

Run No.	Medium	Lot	pH	100°C. (212°F.)		105°C. (221°F.)		110°C. (230°F.)		115°C. (239°F.)		120°C. (248°F.)	
				Surv.	Dest.	Surv.	Dest.	Surv.	Dest.	Surv.	Dest.	Surv.	Dest.
F8	PO..... Asparagus.....	A	7.00	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.
			5.03	39.6	13.6	3.1	3.6	3.1	3.6
F9	PO..... Asparagus.....	A	7.00	24.6	6.8	1.6	2.1	1.6	2.1
			5.03	39.6	14.6	4.1	4.6	4.1	4.6
F11	PO..... Asparagus.....	B	7.00	No end	25.6	7.6	2.1	2.6	2.1	2.6
			5.10	440	point.	160	170	35.6	15.6	4.1	4.6	4.1	4.6
F12	PO..... Asparagus.....	B	7.00	540	180	70	80	31.6	10.6	2.6	3.1	2.6	3.1
			5.10	160	60	70	25.6	9.6	2.6	3.1	2.6	3.1
F13 F14	PO..... Asparagus.....	B	7.00	750	780	160	170	41.6	13.6	5.1	5.6	5.1	5.6
			5.10	200	210	70	80	29.6	11.6	2.6	3.1	2.6	3.1
G5	PO..... Peas, no sugar or salt.....	A	7.00	105	120	10.6
			5.88	105	120	8.6
G7	PO..... Asparagus..... Peas.....	C B	7.00	420	450	110	120	39.6	11.6
			5.43	152	180	50	60	13.6	5.6
H1	PO..... Asparagus..... Peas..... Spinach.....	D C B	5.94	480	510	100	110	30.6	8.6
			7.00	390	420	110	120	33.6	11.6
H2	PO..... Asparagus..... Peas..... Spinach.....	D C B	5.04	120	150	40	50	9.8	5.6
			6.00	450	480	120	140	41.6	11.6
	PO..... Asparagus..... Peas..... Spinach.....	D C B	5.39	240	300	70	80	27.6	8.6
			7.00	45.0	11.6
	PO..... Asparagus..... Peas..... Spinach.....	D C B	5.04	15.6	7.6
			6.00	50.0	10.6
	PO..... Asparagus..... Peas..... Spinach.....	D C B	5.39	35.6	9.6
			7.00	45.0	11.6

TABLE 2

Survival and Destruction Times for Clostridium Botulinum, Strain 62, Type A Subcultured in Tubes

Run No.	Medium	Lot	pH	100°C. (212°F.)		105°C. (221°F.)		110°C. (230°F.)		115°C. (239°F.)	
				Surv.	Dest.	Surv.	Dest.	Surv.	Dest.	Surv.	Dest.
G8	PO ₄		7.00	min.	min.	min.	min.	min.	min.	min.	min.
	Peas.....	C	5.42	160	180	34.6	40.0	5.6	7.6	1.6	2.6
	Spinach.....	A	5.37	160	180	29.6	34.6	3.6	5.6	1.6	2.6
	Asparagus.....	C	5.42	100	120	29.6	34.6	3.6	5.6	1.6	2.6
H4	PO ₄		7.00	80	100	14.6	19.6	3.6	5.6	0.6	1.6
	Peas.....	C	6.00	150	180	11.6	15.6
	Spinach.....	B	5.39	180	210	7.6	11.6
	Asparagus.....	D	5.04	120	150	11.6	15.6

TABLE 3

Survival and Destruction Times for Clostridium Botulinum, Strain 213, Type B Subcultured in Tubes

Run No.	Medium	Lot	pH	100°C. (212°F.)		105°C. (221°F.)		110°C. (230°F.)		115°C. (239°F.)	
				Surv.	Dest.	Surv.	Dest.	Surv.	Dest.	Surv.	Dest.
G6	PO ₄		7.00	min.	min.	min.	min.	min.	min.	min.	min.
	Peas.....	B	5.94	150	180 ¹	29.6 ¹	9.6
	Spinach.....	A	5.37	240	270	40.0	50.0	9.6	13.6
	Asparagus.....	C	5.42 ¹	90 ¹	29.6 ¹	9.6
H4	PO ₄		7.00	120	150	19.6	29.6	6.6	9.6
	Peas.....	C	6.00	210	240	15.6	19.6
	Spinach.....	B	5.39	240	270	24.6	29.6
	Asparagus.....	D	5.04	180	210	11.6	15.6
				90	120	7.6	11.6

¹ No tube positive.

mal death-time curves (Tables 6 to 10). The curves are determined as follows:

1. A survival point is considered as positive data and the curve must be above (higher in temperature or longer in time) every survival point.

2. Destruction points are indicative but not positive, owing to the phenomenon of "skips" (survival of organisms at a time beyond that at which sterility is indicated). In general, however, a thermal death curve should lie beneath as many destruction points as possible and still be above all survival points.

3. The slope of the thermal death-time curve should be parallel to the general trend of the survival and destruction points.

Obviously if the thermal death time has been determined at only two points, for example,

Temperature	Survival	and	Destruction
221°F.	60 min.		74 min.
239°F.	8 min.		10 min.

a number of lines can be drawn thus:

1. Just above the two survival points $F = 2.3$, $z = 20.4$
2. Just below the two destruction points $F = 3.0$, $z = 20.8$
3. Just above one survival and below the other destruction point $F = 3.4$, $z = 23.0$
4. Just above the other survival and below one destruction point $F = 2.2$, $z = 18.9$.

This is illustrated (Fig. 11). The rule followed in obtaining the values (Tables 6 to 11) is illustrated by the dotted line drawn midway between the survival and destruction points. As more points are obtained the line becomes more precisely fixed as to direction and position. In general, as the line is tilted more or less, the values of F and z decrease and increase together. The figures in the tables mentioned accordingly can be varied slightly from those shown.

Runs in which more than three points are obtained yield the most accurate data. For this reason the number of points available in a run is shown in the table. Factors obtained in runs having only two points must be considered approximate, since a failure of an organism to grow at one time influences greatly the slope obtained.

Composite factors for F and z are obtained by plotting the data from all similar runs (same suspension or same organism in the same medium), as shown graphically (Figs. 1 to 9). In general, the greater the number of thermal death points the more closely is the semi-log character of the curve approached.

TABLE 4 (Concluded)

H20	PO ₄	19.6 No growth.	24.6 30.0	1.6	2.1
	Yellow Bantam, canned.....	CC ¹ DD ¹	3.0	3.5
	White, canned.....		3.0	3.5

<i>Cl. botulinum</i> 213B											
Corn in 208 x 006 cans; 11.5 gm. per can + 1.1 c.c. 8.25% NaCl brine. PO ₄ runs in tubes.											
H11	PO ₄	B	60.0	65.0	13.6	15.6	5.6	6.6	1.35
	Vac. pack, whole-grain, canned.....		65.0	75.0	16.0	18.0	6.0	8.0	1.75
H19	PO ₄	C D	40.0	50.0	No growth.	3.1
	Yellow Bantam, raw.....		75.0	80.0	4.0	6.0
	White, raw.....		4.0	5.0
H20	PO ₄	CC ¹ DD ¹	34.6	40.0	1.6	2.1
	Yellow Bantam, canned.....		50.0	60.0	No growth.	3.5
	White, canned.....		No growth.

¹ This corn was from lots C and D, vacuum packed and processed.

TABLE 5

Survival and Destruction Times for Organism No. 3679, and *Clostridium Botulinum* 62A and 213B in Evaporated Milk and Cream

Medium	Total solids	pH	No. 3679—Milk in cans											
			100°C. (212°F.)		105°C. (221°F.)		110°C. (230°F.)		115°C. (239°F.)		120°C. (248°F.)		125°C. (257°F.)	
			Surv.	Dest.	Surv.	Dest.	Surv.	Dest.	Surv.	Dest.	Surv.	Dest.	Surv.	Dest.
<i>Cl. botulinum</i> 62A (in tubes, subcultured)														
Run H9														
PO ₂	7.00	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.
Skin milk.....	17.69	6.45	75.0	105.0	27.6	31.6	8.6	9.6
Whole milk.....	25.61	6.36	60.0	105.0	24.0	32.0	9.0	12.0
Whole + 2.3 gm. per gal. NaHCO ₃	25.69	6.43	30.0	60.0	24.0	32.0	9.0	12.0
Run H12														
PO ₂	7.00	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.
Skin milk.....	19.43	6.26	270	300	90.0	100.0	27.6	31.6	7.6	8.6	2.6	3.1	0.35	0.6
Whole milk.....	26.78	6.24	140	160	50.0	60.0	16.1	20.0	6.25	8.0	2.0	2.5	0.75	1.0
Whole milk.....	32.59	6.19	120	150	40.0	50.0	20.0	24.0	6.25	7.0	2.0	2.5	0.75	1.0
Whole + .625 gm. per liter NaHCO ₃	26.78	6.30	120	150	No growth	10.0	4.5	5.5
Whole + .01% lactic acid.....	26.78	6.29	60.0	70.0	7.0	8.0
Cream, 19.74% fat.....	37.75	6.30	40.0	70.0	10.0	12.0	4.0	5.0
Cream, 19.74% fat ¹	14.0	No end point
<i>Cl. botulinum</i> 213B (in tubes, subcultured)														
Run H10														
PO ₂	7.00	29.6	45.0	7.6	9.6
Whole milk.....	6.15	No growth	29.4	1.4	3.4
Run H11														
PO ₂	7.00	31.6	35.6	9.6	11.6	3.6	4.1
Whole milk.....	26.53	6.34	23.4	27.4	9.4	11.4	1.4	2.4
Run H10														
PO ₂	7.00	60.0	75.0	7.6	9.6
Whole milk.....	6.15	29.4	60.0	1.4	3.4
Run H11														
PO ₂	7.00	60.0	65.0	13.6	15.6	5.6	6.6	1.35	1.6
Whole milk.....	26.53	6.34	50.0	55.0	5.4	9.4	1.4	1.9	0.65	0.9

¹ This lot of cans was incubated at 24°C. (75.2°F.) instead of 37°C. (98.6°F.).

TABLE 6

F and z Values for Organism No. 3679, Suspension No. 3, With 1,200,000 Spores per Container Subcultured in Tubes

Run No.	Medium	Con- tainer	Number of points	F	z
F8.....	PO ₄	Tubes	3	2.70	17.3
F9.....	PO ₄	Tubes	3	3.65	19.6
F11.....	PO ₄	Tubes	4	3.36	17.3
F12.....	PO ₄	Tubes	4	3.18	17.0
F13.....	PO ₄	Tubes	5	4.07	16.8
F8.....	Asparagus	Tubes	3	1.40	16.9
F9.....	Asparagus	Tubes	3	1.78	17.7
F11.....	Asparagus	Tubes	4	2.93	20.0
F12.....	Asparagus	Tubes	4	2.47	20.4
F14.....	Asparagus	Tubes	5	3.30	21.1
Composite.....	PO ₄ (Fig. 2)	Tubes	4.00	16.6
Composite.....	Asparagus	Tubes	3.30	21.1

TABLE 7

F and z Values for Organism No. 3679, Suspension No. 5, With 8,000 Spores per Container Subcultured in Tubes

Run No.	Medium	Con- tainer	Number of points	F	z
G5.....	PO ₄	Tubes	2	2.36	17.3
G7.....	PO ₄	Tubes	4	2.48	16.9
H1.....	PO ₄	Tubes	4	2.68	17.4
H2.....	PO ₄	Tubes	2	2.20	15.6
G5.....	Peas, no salt or sugar	Tubes	2	1.50	15.5
G7.....	Asparagus	Tubes	4	0.95	16.6
H1.....	Asparagus	Tubes	4	1.35	19.0
H2.....	Asparagus	Tubes	2	3.10	30.0
G7.....	Peas	Tubes	4	1.46	15.0
H1.....	Peas	Tubes	4	2.45	16.6
H2.....	Peas	Tubes	2	1.60	13.6
H1.....	Spinach	Tubes	4	1.83	17.6
H2.....	Spinach	Tubes	2	1.90	16.2
Composite.....	PO ₄ (Fig. 3)	Tubes	2.66	16.9
Composite.....	Asparagus	Tubes	1.93	19.8
Composite.....	Peas	Tubes	3.00	16.9
Composite.....	Spinach	Tubes	2.60	18.2

For purposes of comparison, the data of Esty and Meyer (1922) on *Cl. botulinum* in phosphate were corrected in accordance with the findings of Ball, Williams, and Merrill. The new factors for the ideal curve in phosphate were found to be $F=2.45$ and $z=17.6$. The z value is in agreement with that of the present results; the F value is, of course, higher. This is partly due to the remarkable variability of resistance of spores of *Cl. botulinum* and partly to the greater numbers of spores used by Esty and Meyer. The authors used from 10 to 200 million spores per container, while Esty and Meyer's ideal curve is based on 60 thousand million.

DISCUSSION

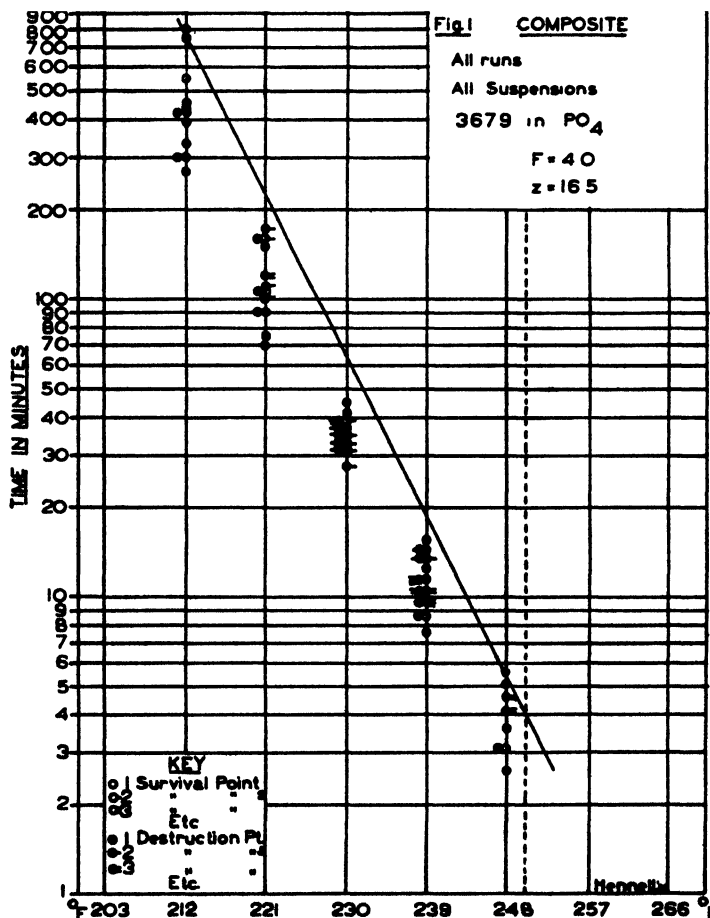
Points of unusually high resistance at one temperature are sometimes encountered; for instance, only in Run F13 with No. 3679, Suspension 3, in phosphate was a destruction point obtained at 100°C. (212°F.), and this is quite high. Also the resistance at 110°C. (230°F. is slightly lower than expected. The results with Suspensions 5 and 6 do not show these peculiarities; however, the influence of this 780-minute point is again seen in the composite curve of all No. 3679 runs in phosphate (Fig. 1).

Another noteworthy point, and one well illustrating the effect of increasing the number of spores used per container, is the survival at 230°F. after 24.6 minutes of *Cl. botulinum* 213B in peas (Run H4), as shown (Fig. 7) along with all the maximum survival points obtained with peas. (In Run H4 only two points were determined.) Giving this point full consideration leads to the line $F=0.87$ and $z=13.6$, which predicts a resistance of 515 minutes at 212°F. in peas. This may appear unreasonable, yet in view of the varying resistance observed at 230°F. (4 to 25 minutes) the percentage change at 212°F. predicted by this line is well within reason. A similar condition exists in the composite of all runs with 62A in phosphate owing to a 7.6-minute survival at 115°C. (239°F.) in Run H10 (Fig. 5).

The z value for No. 3679 is slightly lower than that for *Cl. botulinum* in phosphate, while in foods the z value for No. 3679 is definitely higher than the z value of *Cl. botulinum*. The difference is so great (from three to six degrees F.) that it should be kept in mind when using No. 3679 as a test organism in inoculated packs. This organism is the most suitable yet found, however, as a substitute for *Cl. botulinum*; and processes for canned foods based on spoilage data obtained by the use of suspensions of No. 3679 of a resistance in phosphate equivalent to the maximum for *Cl. botulinum* should be on the safe side for *Cl. botulinum*. The low z values found for *Cl.*

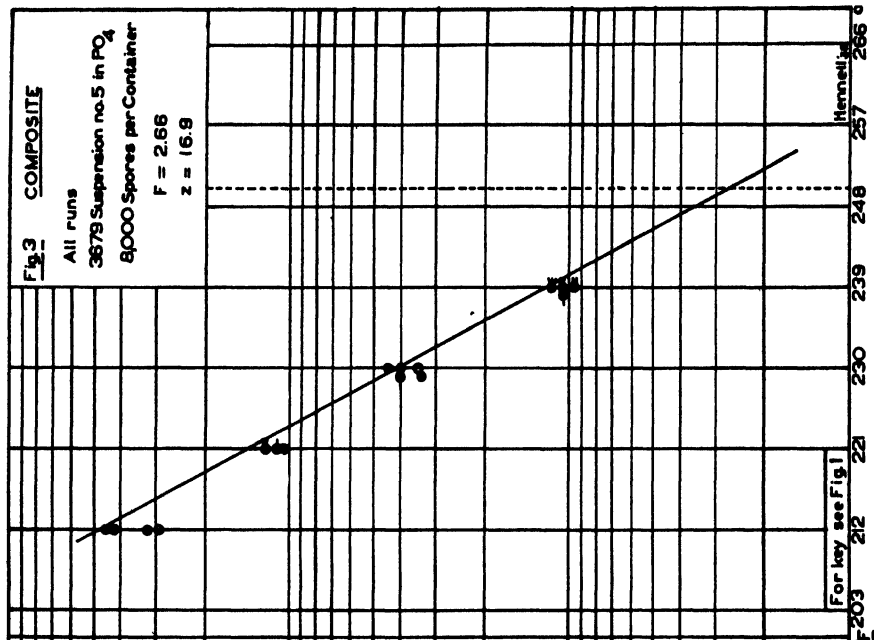
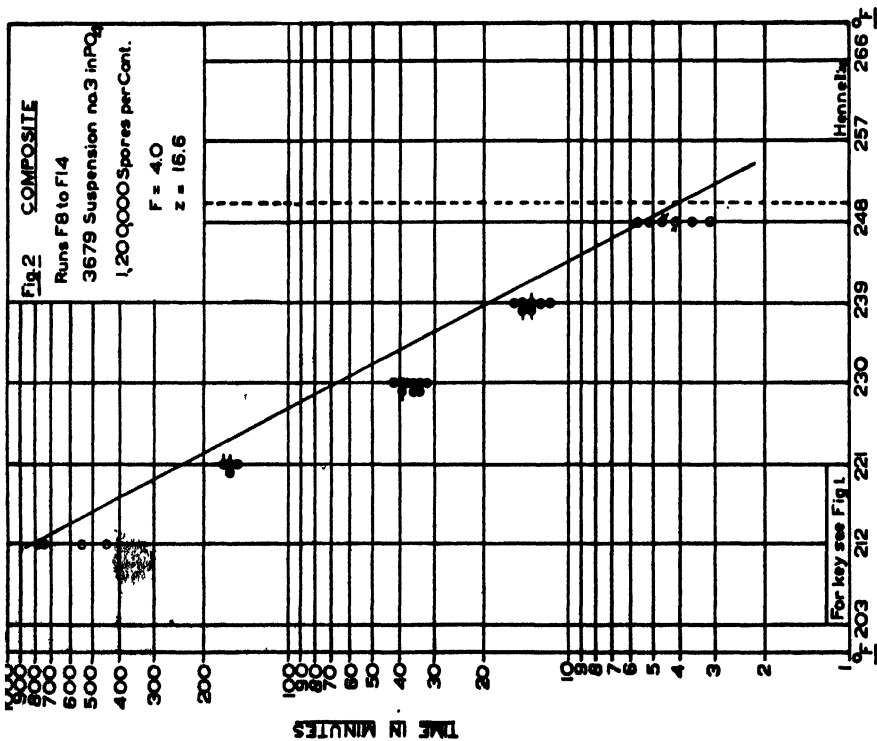
botulinum in foods are of interest in that high-temperature processes (over 240°F.) are indicated as being particularly effective.

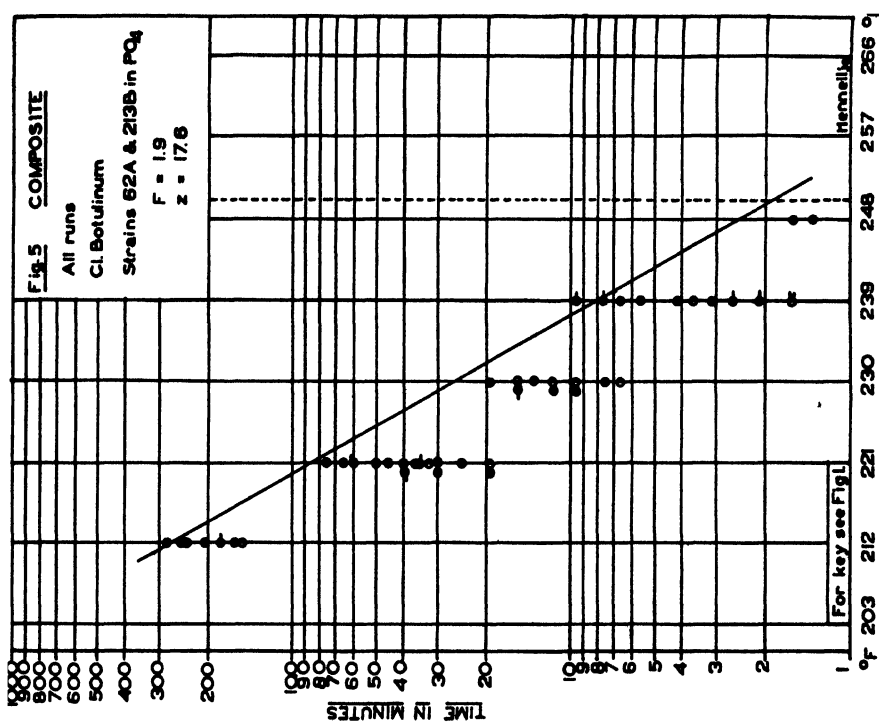
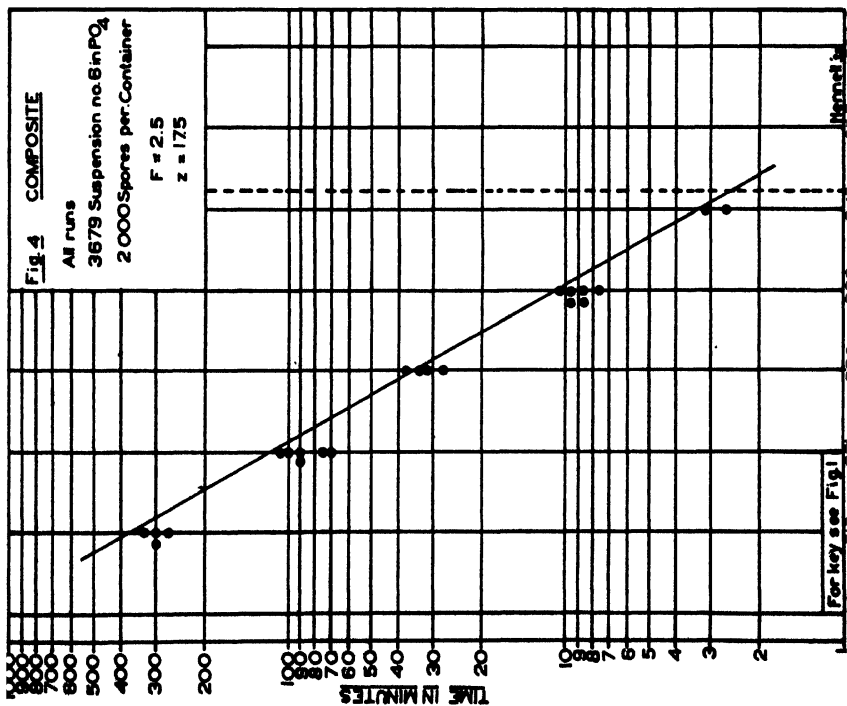
The data on *Cl. botulinum* are given in their entirety so that the variability in results can be emphasized. In a number of runs no growth was obtained at one or more temperatures and in a few runs no spores were viable, yet in the next run this same suspension showed

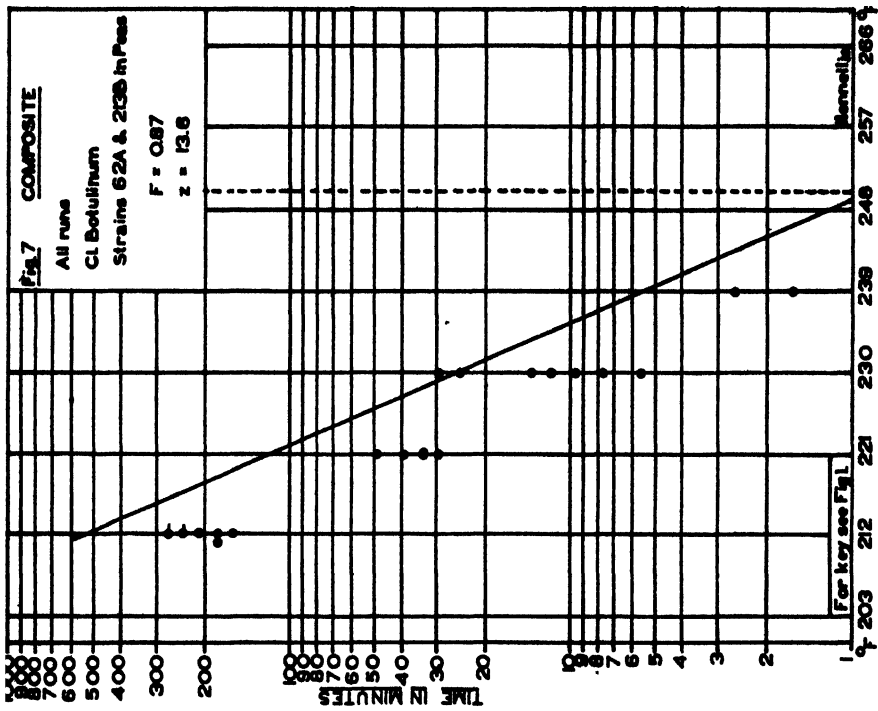
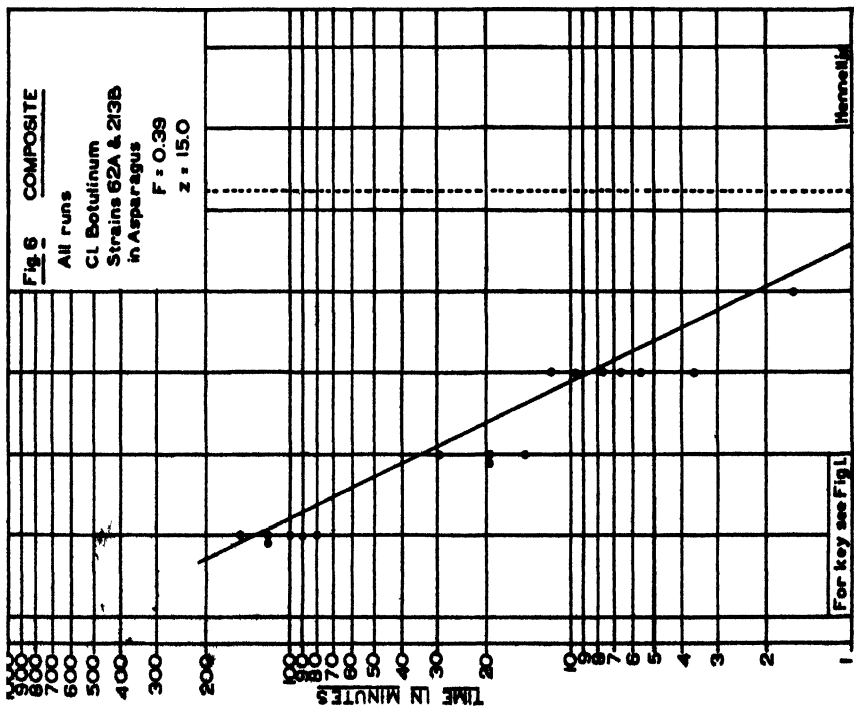


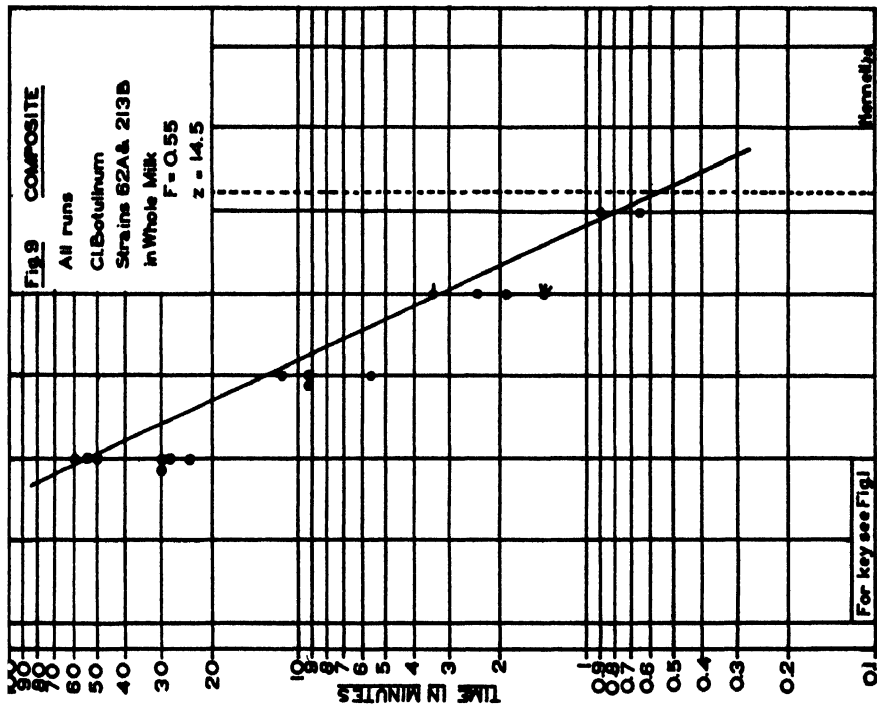
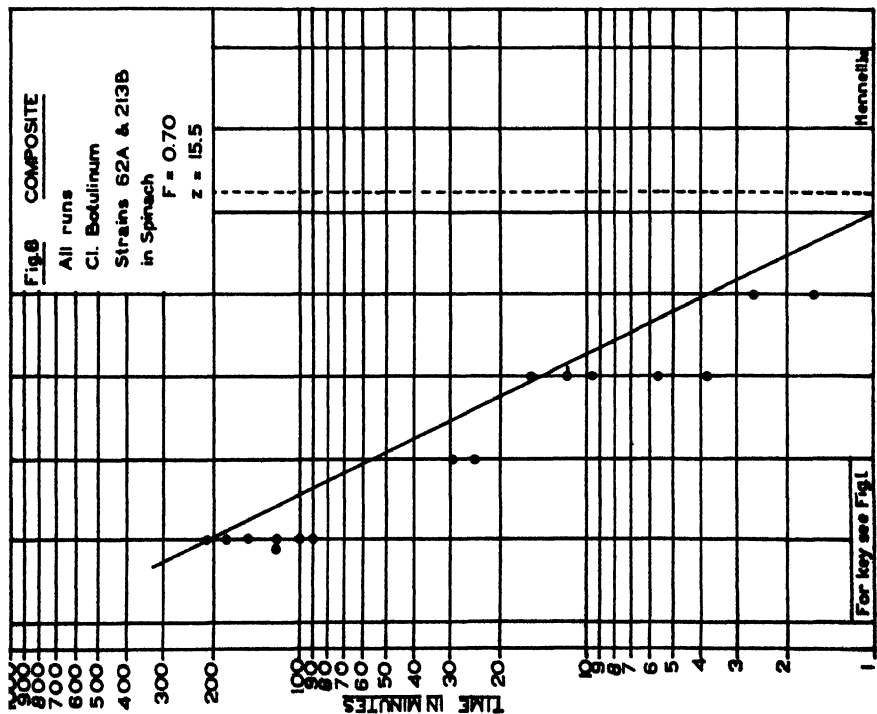
the expected resistance. Under these conditions resistances higher than those found are distinctly possible. In the three green vegetables studied—asparagus, peas, and spinach—*Cl. botulinum* exhibited the greatest resistance in peas, the least in spinach. The differences are definite but not great.

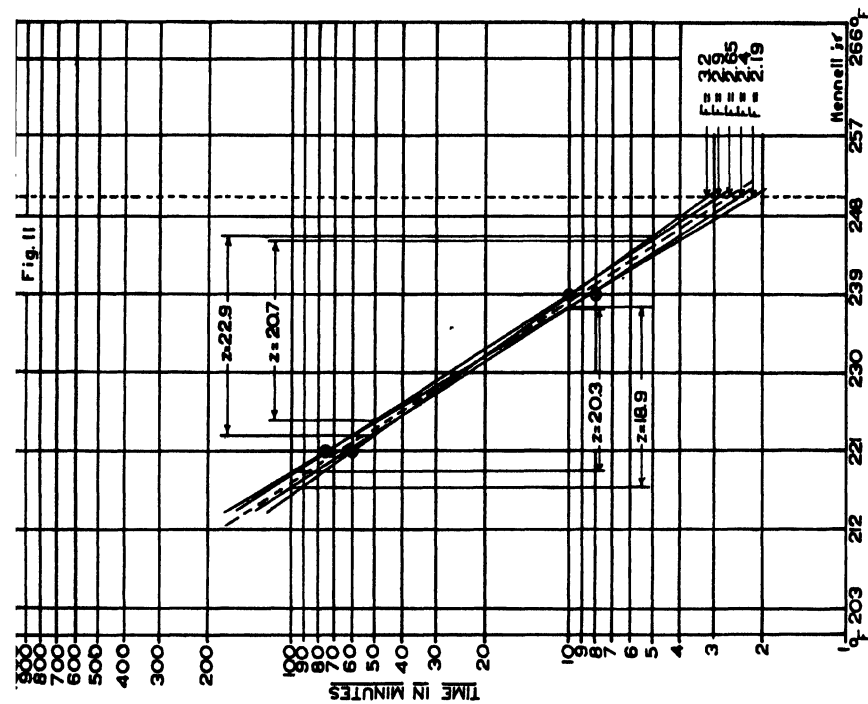
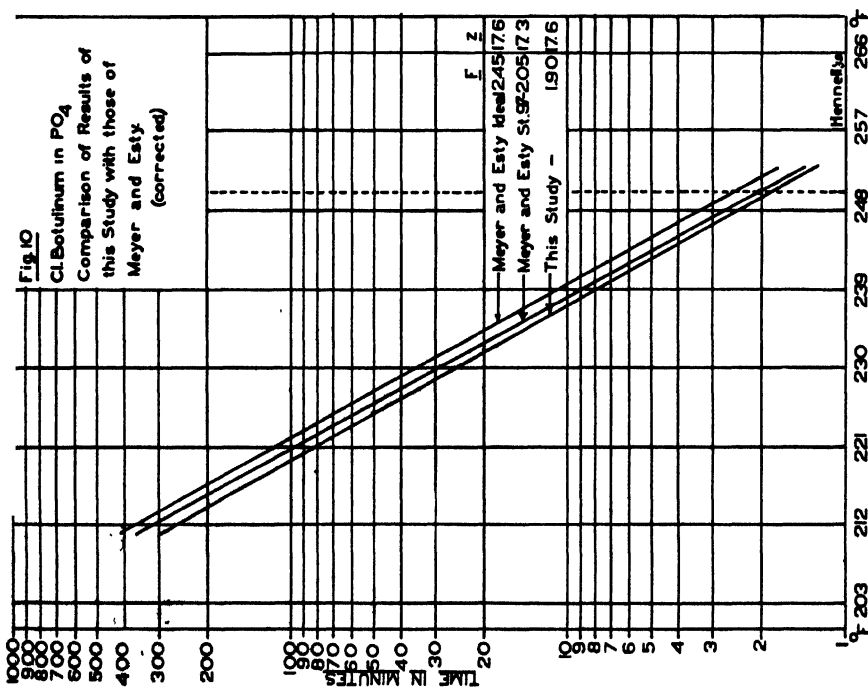
The resistance of *Cl. botulinum* appears to be higher in raw corn than in canned corn.











The runs with No. 3679 in milk indicate that the resistance in whole milk and in skim milk is about the same, while the addition of a small amount of NaHCO_3 raises the resistance somewhat. The resistance in cream is higher than in whole milk, and here the temperature of incubation appears important since a definitely longer

TABLE 8
*F and z Values for Organism No. 3679, Suspension No. 6, With 2,000
Spores per Container*

Run No.	Medium	Con- tainer	Number of points	<i>F</i>	<i>z</i>
H8.....	PO ₄	Tubes	4	2.77	18.5
H9.....	PO ₄	Tubes	3	2.31	18.2
H12.....	PO ₄	Tubes	6	2.05	18.0
H8.....	Vac. pack, whole-grain, canned corn	Cans	4	2.30	19.4
H9.....	Skim milk, 17.69% solids, pH 6.45	Cans	3	2.94	20.4
H12.....	Skim milk, 19.43% solids, pH 6.26	Cans	6	1.76	19.4
H9.....	Whole milk, 25.61% solids, pH 6.36	Cans	3	3.95	25.4
H12.....	Whole milk, 26.78% solids, pH 6.24	Cans	6	1.95	19.5
H9.....	Whole milk + 2.3 gm. per gal. NaHCO ₃ , 25.69% solids, pH 6.43	Cans	4	2.94	20.4
H12.....	Whole milk + .625 gm. per liter NaHCO ₃ , 26.78% solids, pH 6.30	Cans	6	3.05	19.5
H12.....	Whole milk + .01% lactic acid, 26.78% solids, pH 6.29	Cans	2	2.61	22.6
H12.....	Cream, 19.74% fat, 37.75% solids, pH 6.30	Cans	2	3.60	23.1
Composite..	PO ₄ (Fig. 4)	Tubes	2.50	17.5
Composite..	Skim milk	Cans	2.60	20.0
Composite..	Whole milk	Cans	2.60	20.0

survival time was found if the cans were incubated at 24°C. (75.2°F.) rather than 37°C. (98.6°F.) to delay fat separation.

Although the figures given (Table 11) appear reasonably consistent, *F* and *z* values for a given organism in the same food are not necessarily constant, wide variations having been encountered in different thermal death-time runs. Again, marked variations were sometimes observed between Type A and Type B strains of *Cl. botulinum* heated under the same conditions. In this connection the following are some of the important factors influencing the results of different

TABLE 9

F and s Values for Clostridium Botulinum, Strain 62, Type A

Run No.	Medium	Con- tainer	Number of points	F	s
Suspension No. 1 with 10,000,000 spores per container					
G8.....	PO ₄	Tubes	4	0.28	13.6
	Peas	Tubes	4	0.28	13.6
	Spinach	Tubes	4	0.33	15.1
	Asparagus	Tubes	4	0.19	14.3
Suspension No. 1 with 20,000,000 spores per container					
H4.....	PO ₄	Tubes	2	0.82	18.0
	Peas	Tubes	2	0.80	13.5
	Spinach	Tubes	2	1.05	18.0
	Asparagus	Tubes	2	0.52	15.8
Suspension No. 2 with 75,000,000 spores per container					
H10.....	PO ₄	Tubes	2	3.60	27.0
H11.....	PO ₄	Tubes	3	1.00	19.0
H20.....	PO ₄	Tubes	2	0.40	16.5
H11.....	Vac.-pack, whole-grain, canned corn	Cans	3	1.79	20.8
H19.....	Yellow Bantam corn, raw	Cans	2	1.14	18.5
H11.....	Whole milk	Tubes	3	0.59	16.1

TABLE 10

F and s Values for Clostridium Botulinum, Strain 213, Type B

Run No.	Medium	Con- tainer	Number of points	F	s
Suspension No. 1 with 12,000,000 spores per container					
G6.....	Peas	Tubes	3	0.38	13.4
	Asparagus	Tubes	3	0.27	14.2
Suspension No. 1 with 200,000,000 spores per container					
H4.....	PO ₄	Tubes	2	1.07	16.3
	Peas	Tubes	2	2.15	18.2
	Spinach	Tubes	2	0.68	15.5
	Asparagus	Tubes	2	0.76	17.5
Suspension No. 2 with 25,000,000 spores per container					
H10.....	PO ₄	Tubes	2	2.53	20.4
H11.....	PO ₄	Tubes	4	1.38	17.5
H20.....	PO ₄	Tubes	2	0.80	13.8
H10.....	Whole milk	Tubes	2	0.42	14.2
H11.....	Whole milk	Tubes	4	0.51	14.4
H11.....	Vac.-pack, whole-grain, canned corn	Cans	4	1.38	17.2
H19.....	Yellow Bantam corn, raw	Cans	2	0.90	14.8

thermal death-time runs with the same organism in the same medium:

1. Variations in the resistance of individual spores in a given suspension.

2. Change in the resistance of spores in a stock suspension on storage.

3. Loss of a portion of the suspension in transferring from the heated container to the enrichment medium.

4. Variations in different lots of the same enrichment medium affecting its suitability for the germination of spores. It has been generally observed that when spores are heated in phosphate and a food juice in the same run and subcultured, the spores heated in

TABLE 11
Comparison of Composite F and z Values for All Runs and All Suspensions

Medium	No. 3679		<i>Cl. botulinum</i> 62A		<i>Cl. botulinum</i> 213B	
	F	z	F	z	F	z
PO.....	4.00	16.6	1.70	16.4	2.00	18.0
Asparagus.....	3.30	21.1	0.39	15.0	0.39	15.0
Peas.....	3.00	16.9	0.30	13.4	1.40	15.6
Spinach.....	2.60	18.2	0.65	15.5	0.68	15.5
Whole milk.....	2.60	20.0	0.45	14.7	0.50	14.3

the food juice germinate much more rapidly than those heated in phosphate. Two c.c. of standard phosphate solution added to a tube of enrichment medium appears to have a definitely inhibiting effect on the germination of spores.

5. Possible lack of germination of a few surviving spores within the period of incubation. With many microorganisms a certain population is necessary for demonstrable growth or even germination to occur. This may account for "skips" (survival at a time beyond that at which sterility is indicated).

In deciding on F and z values for a given food, therefore, caution must be used and due allowance must be made for possible variations in determining a safe process. Considerable further work is necessary to determine the factors F and z for *Cl. botulinum* in various foods, with special reference to raw rather than to processed products. In this future work it is strongly urged that at least five thermal death-time points be determined at each run. In the selection of the time intervals at each temperature the following series is suggested. The numbers in this series are approximately the same logarithmic distance apart (.05, or one-twentieth, of the log cycle). Decimal fractions or multiples of these numbers should be used to extend the range to that needed. To reduce the number of points, if necessary, (this

will lessen the precision of the data, however) select numbers at regular intervals from this series:

1.0	2.0	4.0	8.0
1.1	2.2	4.4	8.8
1.25	2.5	5.0	10.0
1.4	2.8	5.6	(11.0)
1.6	3.2	6.4	(12.5)
1.8	3.6	7.2	(14.0)

CONCLUSIONS

1. The heat-resistance characteristics of *Cl. botulinum* in canned foods are different from those in neutral phosphate.
2. The z values of *Cl. botulinum* in food media apparently lie in the range 13.4 to 15.6, as contrasted with the range 16.4 to 18.0 for neutral phosphate.
3. The heat-resistance characteristics of Cameron's organism No. 3679 are unlike those of *Cl. botulinum* in food media but are nearly similar in neutral phosphate. The z values of No. 3679 are higher in food media than the z values of *Cl. botulinum*.
4. The work of Esty and Meyer on *Cl. botulinum* in neutral phosphate is well substantiated.
5. The corrected factors for the ideal thermal death curve of *Cl. botulinum* in neutral phosphate as determined by Esty and Meyer are $F = 2.45$ and $z = 17.6$.

ACKNOWLEDGMENT

The authors wish to acknowledge their indebtedness to Paul C. Wilbur for valuable assistance in the work on corn and evaporated milk while he was connected with the Research Department of the American Can Company; to Dr. C. O. Ball of the same department for critical analysis of the data and for his valuable suggestions; and also to E. Wagner Sommer, of the Hooper Foundation for Medical Research, University of California, for her work in preparing all the suspensions of *Cl. botulinum* spores used in these experiments.

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SALMONELLA FOOD POISONING—INFECTION OR INTOXICATION?

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Savage and White (1925a, 1925b) have brought forth evidence to implicate toxic factors produced by the growth of *Salmonella* organisms as responsible for certain outbreaks of food poisoning in which living *Salmonella* bacteria could not be demonstrated. In their experimental work (1925a) they found that a substance was produced by the bacteria which was definitely toxic to laboratory animals if injected intravenously or intraperitoneally. This has been confirmed since that time by many workers: Dack, Cary, and Harmon (1928); Branham (1925); Bahr and Dyssegaard (1927). Geiger and Meyer (1928) and Van Ermengem (1892) reported gastro-intestinal symptoms and death from feeding laboratory animals an apparently similar preparation; but Dack, Harmon, and Jarra (1928), Dack, Cary, and Harmon (1928), Verder and Sutton (1933), and Bahr and Dyssegaard (1927) reported no gastro-intestinal disturbance at all from feeding. Savage and White (1925b) described an irritation so mild that rabbits must be killed and their stomach mucosa examined to observe the reaction. Branham, Robey, and Day (1928) described another *Salmonella* product which is toxic when fed to mice, but it is apparently not the factor responsible in the above cases or in human food poisoning.

In the determination of causative organisms in cases of food poisoning where *Salmonella* was suspected but no living organisms could be found in the food, Savage and White (1925b) tested for the presence of specific toxic substances by the following three methods:

1. Production of gastro-intestinal irritation in rabbits fed suspected food.
2. Demonstration of specific agglutinins in patients' serum.
3. Injecting animals with extracts of the suspected food and demonstrating specific agglutinins in their serum.

The first method consisted of feeding suspected food to young rabbits, killing them in nine to twelve hours, and examining the stomach and duodenum. Post-mortem examination showed various degrees of hyperemia of mucosa with little correlation between severity of hyperemia and dosage, strain, etc. The method of killing the rabbits

was not reported. No controls, fed other materials and killed in the same way after the same interval, were reported. These experiments have been repeated with negative results by Dack, Harmon, and Jarra (1928), on 17 rabbits killed by suffocation.

Savage and White state that their second criterion is not very reliable. The agglutination titers were all very low (only two above 1:100) and in many cases negative. Where there was definite agglutination the titer varied considerably from one day to the next. They explain the lack of agglutinins in some patients by the rapid expulsion of antigen owing to the diarrhea and vomiting, and in others by the ingestion of the toxic food after it had been well cooked; since the same authors (1925a) could demonstrate no agglutinins in laboratory animals which had been fed boiled cultures.

The results from the experiments based on the third of Savage and White's criteria can be interpreted differently. Owing to the prevalence of *Salmonella* infections in domestic animals and rodents, the possibility exists that the animals may have had slight infections in the past, leaving a low-titer antibody reserve. This may increase after injection of a foreign antigen, such as the suspected food, since any intravenous injection of protein, bland or toxic, increases the titer of all antibodies present in the serum (anamnestic phenomenon). No titer reported here (1925b) was higher than 1:200; the majority were much lower, and no control with bland protein was recorded.

Since the toxic substance affected human beings and laboratory animals so differently and involved an organism differing so much in pathogenicity for the two groups, the work was checked with human volunteers. Savage and White tested heat-killed *Salmonella schottmüller*i by ingestion and got no gastro-intestinal disturbance. They make the following statement (1925a): "From experience with food poisoning outbreaks there can be no doubt that the human stomach would be definitely affected, and probably markedly so if *B. aertrycke* in similar quantity had been consumed. This experiment was not attempted." Dack, Cary, and Harmon (1928) fed 24 human volunteers with killed cultures of *Salmonella aertrycke* and *S. enteritidis*, and elicited no harmful symptoms of any sort. They found no agglutinins in the blood of these experimental subjects tested 10 days after feeding.

Verder and Sutton (1933) contaminated sterile custards with *S. enteritidis*, incubated them for 24 hours, then killed the organisms by thorough heating. This custard, when fed to human volunteers, produced no symptoms. In one case, however, in which the organisms in the custard were not all killed (checked by sterility test at the time of ingestion) the volunteer became violently ill with a case of typical

food poisoning, and *S. enteritidis* was recovered from his stool. Monkey feedings with living *Salmonella* were definitely positive—Dack, Jordan, and Wood (1929).

In recent investigations of food poisoning in Austria, Hammerschmidt (1935) drew the definite conclusion that outbreaks are due to infections with salmonellae from food or, in a few cases, by contact with persons actually ill with food poisoning. Bahr (1928) drew the same definite conclusion from his experiments in Copenhagen. The evidence at the present time is overwhelmingly in favor of infection as the cause of *Salmonella* food poisoning.

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A STUDY OF CERTAIN REDUCING SUBSTANCES IN CITRUS FRUITS ¹

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On application of the Thunberg technique ² (1916) to the juices of orange, lemon, and grapefruit, the methylene blue was found to be rapidly reduced, which indicated the presence of one or more reducing substances. Studies were then made in an effort to establish the identity of the reducing substance, or substances, and to determine the distribution in the juice, pulp, and peel of citrus fruits. The main reducing substances known to occur in foods are ascorbic acid and glutathione. That ascorbic acid is abundant in citrus fruits is known, but the concentration of glutathione in such fruits received little study, which problem has been studied in this investigation. Another phase of this research deals with the influence of various factors, such as ultra-violet irradiation, acidity, and alkalinity, on the stability of ascorbic acid in citrus fruits.

EXPERIMENTAL PROCEDURE

The reactions were carried out in special test tubes (Thunberg tubes) which were fitted with a ground glass stopper, a side arm to permit evacuation, and a side cup to permit the introduction of a second liquid without mixing with the first. Four ml. of the juice were placed in the main body of the tube, and one ml. of a 1:5,000 solution of methylene blue was placed in the side cup. The tube was then evacuated to remove all oxygen and allowed to come to thermal equilibrium in a water bath at 37.5°C. (99°F.). It was then shaken to thoroughly mix the two solutions; and the time required for the reduction of the methylene blue, as shown by the disappearance of color, was noted. Preliminary investigations showed that the time for reduction of the dye was directly proportional to its concentration and inversely proportional to the concentration of a reducing agent such as ascorbic acid. Since this latter substance is a known component of the juice, and is known to be capable of the anaerobic reduction of methylene blue, its concentration was separately determined by titration with 2,6-dichlorophenolindophenol according to the method of Birch, Harris, and Ray (1933). Aliquot samples of

¹ Research paper No. 457, Journal Series, University of Arkansas.

² This technique is described in the next paragraph.

TABLE 1

(a) *Effect of Ultra-Violet Irradiation Upon Stability of Reducing Substances in Orange Juice, as Shown by Time for Reduction of Methylene Blue*

Period of irradiation (minutes).....	1	2	3	4	5	10	15	30	45	60	120
Initial ascorbic acid (mg. per c.c.).....	.84	.84	.84	.84	.84	.84	.84	.84	.84	.84	.84
Final ascorbic acid (mg. per c.c.).....	.61	.50	.40	.30	.28	.15	.12	.02	.01	0	0
Initial period required for reduction of methylene blue (minutes).....	14	14	14	14	14	14	14	14	14	14	14
Final period required for reduction of methylene blue (minutes).....	17	22	26	37	48	68	— ¹	—	—	—	—

(b) *Effect of Ultra-Violet Irradiation Upon Stability of Pure Ascorbic Acid, as Shown by Time for Reduction of Methylene Blue*

Period of irradiation (minutes).....	0	5	10	15	20	25	30	40	45	60	120
Ascorbic acid (mg. per c.c.).....	.50	.49	.42	.40	.30	.23	.13	.12	.10	.08	.001
Period required for reduction of methylene blue (minutes).....	4	5	11	13	21	25	27	35	43	56	— ¹

(c) *Effect of Irradiation of Orange Juice Followed by Reduction With H₂S, as Shown by Time for Reduction of Methylene Blue*

Original juice		Irradiated juice		Irradiated juice treated with H ₂ S	
Ascorbic acid	Reduction time	Ascorbic acid	Reduction time	Ascorbic acid	Reduction time
mg. per c.c.	min.	mg per c.c	min.	mg. per c.c.	min.
.849	14	.03	— ¹	.827	16
.786	14	.01	—	.738	19
.827	14	0	—	.802	16

¹ The dash indicates that no reduction of methylene blue took place even after contact with the ascorbic acid solution for several hours.

the juice were taken, diluted to a total volume of five ml., and titrated with a .04-per cent solution of the dye. A microburette was used for the titration, which was completed in two minutes or less, according to the recommendations of Ahmad (1935).

As ascorbic acid is very readily oxidized, its presence in the reduced form suggests the existence of mechanisms inhibiting oxidation, or of other reducing substances. Barron, Barron, and Klemperer (1936) suggest that glutathione may serve in some instances to maintain ascorbic acid in the reduced state. Titration for this substance was made according to the method of Okuda and Ogawa (1933). The sample was ground in a mortar with five ml. of N/2 sulfosalicylic acid, the excess liquid decanted, and the grinding repeated with an additional five ml. of the acid. The total volume was then made up to 50 ml. by the addition of N/4 sulfosalicylic acid, and the material shaken at intervals for 30 minutes. It was then centrifuged for 10 minutes at 1,050 r.p.m., the supernatant liquid decanted, and the titration for glutathione carried out at 0°C. (32°F.) with a M/10,000 solution of KIO_3 . The ascorbic acid content of the extract was determined by titration with 2,6-dichlorophenolindophenol, and the difference between the total titer and the KIO_3 equivalent to the ascorbic acid was taken as representing the glutathione content.

The differentiation of the reducing substances was attempted by selective oxidation. In this manner it was hoped to remove the ascorbic acid and leave any other substances intact. Copper, being known to catalyze the oxidation of ascorbic acid in the presence of air, the juice was treated with anhydrous copper sulfate to yield a final concentration of copper ion of one part in five thousand. After the solution had been stirred for 30 minutes the ascorbic acid titer and the reaction with methylene blue were both destroyed.

Von Euler and Malm (1932-1933) suggest that ascorbic acid may be destroyed by the formation of a cyanohydrin with aqueous KCN. When fresh orange or lemon juice was mixed with an equal volume of five-per cent aqueous solution of KCN, the ascorbic acid titer and the methylene blue were both destroyed.

The effect of ultra-violet light upon the reducing substances in citrus-fruit juices was determined by exposing a two mm. layer of the fresh juice to the rays of a Cooper-Hewitt "Uviarc" mercury vapor lamp having a conical metal reflector and drawing four amperes at 200 volts D. C. The distance from the lamp was 42 cm. The period of irradiation varied from one minute to two hours. The ascorbic acid content and the speed of the reaction with methylene blue decreased rapidly with an increase in the time of irradiation (Table 1). That this decrease is due to the transformation of the

TABLE 2

(a) Effect of pH Upon Stability of Reducing Substances in Orange Juice, as Shown by Period Required for Reduction of Methylene Blue

pH.....	3.2	3.6	3.9	4.7	5.1	5.4	5.5	5.6	5.7	6.0	6.3	6.4	6.5	6.7	6.9	7.3	8.5	9.0	9.5
Ascorbic acid (mg. per t.e.).....	.85	.85	.75	.52	.35	.20	.12	.09	.04	.02	.01	0	0	0	0	0	0	0	0
Period required for reduction of methylene blue (minutes)....	15	18	24	50	65	75	84	85	84	70	55	45	45	42	31	27	6	2	1

(b) Effect of pH Upon Stability of Pure Ascorbic Acid, as Shown by Period Required for Reduction of Methylene Blue

pH.....	3.0	3.6	3.9	4.2	4.3	4.6	5.1	6.2	7.4	8.3	9.0	9.5
Period required for reduction of methylene blue (minutes).....	5	14	24	37	49	57	71	— ²	—	—	—	—

(c) Effect of pH Upon Sugars, as Shown by Period Required for Reduction of Methylene Blue (Solution containing 3% glucose, 3% fructose, and 6% sucrose)

pH.....	4.0	5.4	6.6	7.7	8.1	8.6	8.9	9.0	9.5
Period required for reduction of methylene blue (minutes).....	— ²	—	—	—	69	39	20	4	1

(d) Effect of pH Upon a Solution of 3% Glucose, 3% Fructose, 6% Sucrose and .05% Ascorbic Acid, as Shown by Period Required for Reduction of Methylene Blue

pH.....	2.9	3.4	3.5	3.6	3.9	4.1	4.5	4.6	5.3	5.8	6.3	6.4	7.0	8.1	9.0	9.5
Period required for reduction of methylene blue (minutes).....	5	20	22	24	39	40	50	58	69	— ²	—	—	76	40	19	1

¹ Titer with 2,6-dichlorophenolindophenol became zero above pH 6.5. ² The dash indicates that no reduction of methylene blue took place even after contact with the ascorbic acid solution for several hours.

l-ascorbic acid to its reversibly oxidized form, the dehydro-ascorbic acid, was shown by the restoration of some 80 per cent of the original titer following reduction of the irradiated solution with zinc dust, or H_2S . Treatment of the non-irradiated juice with these reagents had no effect upon the ascorbic acid titer, or upon the speed of the reaction with methylene blue. Solutions of pure crystalline ascorbic acid showed similar behavior on irradiation followed by reduction with H_2S . The work of Kon and Watson (1936), which appeared during this investigation, confirms the statement as to the oxidative nature of the destruction of ascorbic acid by ultra-violet irradiation.

An increase in the acidity of the juice failed to produce any change in the reducing properties. An increase in the alkalinity produced a rapid decrease in the ascorbic acid titer and in the speed of the reaction with methylene blue. Virtually all the ascorbic acid had disappeared at a pH of 5.6, and the time for the methylene blue reaction reached a maximum at this point. Above this level the speed of the methylene blue reduction increased rapidly with an increase in pH, becoming almost instantaneous above pH 9.3. This formation of a highly reducing solution at the higher pH levels suggests the "reductone" of von Euler, Mayerbäch, and Larson (1933). Control solutions were prepared, consisting of ascorbic acid alone; of a mixture of glucose, fructose, and sucrose in approximately the same concentration as in orange juice; and of these three sugars plus ascorbic acid. Ascorbic acid is destroyed at the higher pH levels (Table 2), while the sugars are inactive under acid conditions but become exceedingly active in strongly alkaline solutions. The behavior of the third solution indicates that ascorbic acid is the principal component of the citrus juices responsible for the reduction of methylene blue at the natural pH of the juice, while the sugars are responsible for the increased activity in alkaline solutions.

The total reducing substances present in the peel and pulp as well as the juice of the orange, lemon, and grapefruit were determined by titration with KIO_3 . The material was extracted with eight-per cent trichloroacetic acid containing four-per cent metaphosphoric acid. The ascorbic acid content was determined by titration with 2,6-dichlorophenolindophenol; the KIO_3 equivalent of the ascorbic acid was determined stoichiometrically and subtracted from the total KIO_3 titer. A second set of samples was taken, using N/2 sulfosalicylic acid as the extractant. In each case the total titer was almost identical with the titer for ascorbic acid (Table 3). It may then be concluded that glutathione is absent and that ascorbic acid is the sole reducing component of the extracts.

TABLE 3
Distribution of Reducing Substances in Citrus Fruits

Fruit	Trichloroacetic acid extract			Sulfosalicylic acid extract		
	Ascorbic acid mg. ¹	KIO ₃ equivalent to ascorbic acid ml. ²	KIO ₃ used ml.	Ascorbic acid mg.	KIO ₃ equivalent to ascorbic acid ml.	KIO ₃ used ml.
Orange	Juice.....	0.823	16.10	16.30	12.55	12.75
	Pulp.....	0.632	12.40	12.85	9.75	10.05
	Peel.....	1.930	37.85	37.95	26.60	27.05
Lemon	Juice.....	0.387	7.60	7.85	5.55	5.90
	Pulp.....	0.321	6.30	6.35	4.70	4.95
	Peel.....	1.010	19.75	20.10	13.50	13.65
Grapefruit	Juice.....	0.374	7.35	7.60	6.00	6.40
	Pulp.....	0.373	7.30	7.45	5.70	5.95
	Peel.....	2.650	52.00	53.00	39.80	41.20

¹ Ascorbic acid expressed in mg. per gm. of sample weight. ² KIO₃ expressed as ml. of M/10,000 solution.

The effectiveness of various solvents in the extraction of ascorbic acid from the peel of citrus fruits was determined, using water, ethyl alcohol, acetone, trichloroacetic acid, sulfosalicylic acid, n-butyl alcohol, and ether as extractants. One gram of the fresh peel was thoroughly ground in a mortar with five ml. of the solvents. At the end of five minutes the excess liquid was decanted and the extraction repeated with an additional five ml. of solvent. Three such extractions were made, the entire contents of the mortar were added to the decanted liquid in a centrifuge tube, the tube was shaken vigorously for two minutes and centrifuged at 1,050 r.p.m. for 10 minutes. The supernatant liquid was decanted, 15 ml. of fresh solvent were added, the mixture was shaken for two minutes and centrifuged. The supernatant liquid was added to the volume first obtained. Further extrac-

TABLE 4
*Extraction of Ascorbic Acid From Orange Peel*¹

Solvent	H ₂ O at 25°C. (77°F.)	H ₂ O at 65°C. (149°F.)	80 per cent C ₂ H ₅ OH at 25°C.	80 per cent C ₂ H ₅ OH at 65°C.
Ascorbic acid (mg. per gm.).....	.32	.16	.73	.39

Solvent	Acetone at 25°C.	Acetone at 60°C. (140°F.)	8 per cent trichloro- acetic acid	n-Butyl alcohol
Ascorbic acid (mg. per gm.).....	.87	.63	1.20	.18

¹ Similar results were obtained with peel from lemon and grapefruit.

tions failed to add to the total amount of ascorbic acid extracted. It was found that trichloroacetic acid was the most effective solvent, followed closely by sulfosalicylic acid and acetone (Table 4).

SUMMARY

Studies were conducted in order to determine the identity of the component of citrus-fruit juices responsible for the reduction of methylene blue according to the Thunberg technique. It was found that the concentration of reducing substances was proportional to the concentration of ascorbic acid, and that all reducing properties were destroyed by reactions destroying ascorbic acid. It may be concluded that ascorbic acid is the sole component which reduces methylene blue under the experimental conditions.

The efficiency of various solvents in the extraction of ascorbic acid from the peel of citrus fruits was also determined. It was found that an eight-per cent solution of trichloroacetic acid was the most effective.

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IODINE CONTENT OF SOME OHIO VEGETABLES

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Marine, Lenhart, and Kimball (1923) have shown that simple goiter is prevalent among school children, especially girls, in the Great Lakes area. Unless remedial measures are taken, the incidence of goiter frequently exceeds 30 per cent of female pupils in the grade schools of this region. These investigators demonstrated that iodine at the rate of 300 milligrams per year is effective in the control of a large percentage of simple goiter. It is obviously of considerable interest to know whether or not vegetables grown under glass and outdoors in this area contain appreciable amounts of iodine. It is also of interest to learn if the iodine content of vegetables can be increased by iodine applications to the soil.

EXPERIMENTAL PROCEDURE

The analytical apparatus for the experiment was similar to that used by Mack and Brasher (1936) except for modifications of the absorption train. The train included in order: a water-jacketed condenser, absorption towers filled with glass beads saturated with potassium carbonate alternated with absorption tubes filled with a 20-per cent solution of sodium hydroxide.

The smoke was cooled and water was condensed in the water-jacketed condenser. Any iodine that was in the smoke was dissolved by the potassium carbonate on the glass beads or in the sodium hydroxide. The absorption train was easily washed with hot water.

The material to be analyzed for the iodine content was dried in a constant temperature oven at 21.1°C. (70°F.). After drying, the samples were ground in an ordinary feed grinder and stored in glass bottles in the oven to prevent them from absorbing water. If the samples were damp while burning, the combustion was irregular and the combustion flask was likely to explode.

A given weight of the material was mixed with two to five grams of powdered calcium carbonate and wrapped in a paper cartridge. This was placed in the combustion tube and burned in an atmosphere of oxygen according to Mack and Brasher's procedure. The combustion apparatus and absorption train were washed with hot water. The washings and ash were boiled down in a liter beaker, transferred to an evaporating dish, and dried. Water was added until a pasty

consistency was reached. It was then extracted with iodine-free, absolute alcohol. The alcohol was evaporated and taken up with water. Iodine was determined by Groak's (1934) method as follows: The solution was neutralized until acid to litmus after which one c.c. of saturated potassium carbonate solution was added. About one c.c. (excess) of N/10 potassium permanganate was added and the solution heated in a water bath for three minutes. Three c.c. of C.P. concentrated phosphoric acid and one c.c. of .33 per cent solution sodium nitrite were added and the mixture heated for one minute in a water bath. The solution was heated again for two minutes following the addition of three c.c. of a 20-per cent solution of urea. Finally five c.c. saturated solution KI were added and titrated with .001 N sodium-thio-sulfate solution. The dead-stop, end-point method

TABLE 1
Comparison of Frear's and Groak's Methods for Determination of Iodine Content

Sample	Dry wt.	Analysis p.p.b. ¹	
		Frear's	Groak's
	gm.		
No. 1 Judy Bros.—Dover.....	5	7,720	5,460
No. 2 Yoder Bros.—Barberton.....	5	12,150	12,310
No. 3 O.S.U.—Columbus.....	5	420	441

¹ All analyses calculated as parts per billion (p.p.b.) of the moisture-free sample.

was used for the titration, but for ordinary results starch was a satisfactory indicator. Comparisons between this method and Frear's (1934) showed little difference in identical samples (Table 1).

LEAF LETTUCE ANALYSES

During the first analyses a 20-gram sample of lettuce was used, but it was evident that such a large sample was not necessary; therefore, the weight of the sample used was reduced to five grams. A smaller sample allowed more check determinations on each specimen. At least two analyses were run on each sample, and if these varied, another was run to check the determination.

Lettuce samples were gathered from several important greenhouse districts in Ohio and analyzed. The lettuce from the greenhouse district around Columbus had the lowest iodine content, having only about 1,500 parts per billion. Samples from Barberton ran almost ten times as great in iodine content (Table 2).

HEAD LETTUCE ANALYSES

A sample of head lettuce was secured from the Imperial Valley in California and analyzed. Samples were also secured from treated

plots at the Experimental Farm of Ohio State University on fertilized plots and on check plots. Analyses of these samples show that the iodine content of Ohio head lettuce on well-fertilized plots is about the same as California lettuce. The sample treated with KI had an increase comparable with the leaf lettuce grown in the greenhouse

TABLE 2
Iodine Content of Leaf Lettuce Grown in Ohio Greenhouses

Area	Iodine ¹ — dry basis	Area	Iodine— dry basis
Columbus—	1,590	Toledo—	8,940
Clarence Salzgeber	2,050	Searles Bros.	10,080
	1,420		
Vermilion—	6,460	Dover—	5,460
Ruetenik Gardens	7,680	Judy Bros.	7,720
	5,460		6,720
Barberton—	12,150	Newark—	6,210
Yoder Bros.	12,510	Warren S. Weiant	6,800
	12,310	& Sons, Inc.	6,300
Cincinnati—	7,560	Ashtabula—	4,660
Herman Meyer	7,180	Frank K. Luce	4,480
	7,980	& Son	

¹ All analyses calculated as parts per billion (p.p.b.) of the moisture-free sample.

(Table 4). The application of 20 pounds of potassium iodide (KI) per acre caused the plants to wilt during the middle of the day but no burning resulted. The sample from a plot receiving 1,000 pounds per acre of a 6-8-6 fertilizer was lower in iodine content than the other samples analyzed (Table 3).

TABLE 3
Analyses of Head Lettuce

Treatment	Iodine ¹ — dry basis
California.....	6,740
Ohio—Fertilized 6-8-6 1000 lbs. per acre.....	4,620
Ohio—Fertilized 6-8-6 plus 63 trace elements at rate of 480 lbs. per acre applied three days before plants were set in field.....	6,300
Ohio—KI at rate of 20 lbs. per acre (center).....	91,560
Applied four days before harvest (outside).....	86,100

¹ All analyses calculated as parts per billion (p.p.b.) of the moisture-free sample.

EXPERIMENTS WITH LEAF LETTUCE FERTILIZED WITH IODINE

Experiments involving attempts to increase the content of iodine in lettuce were successful. Iodine in the form of KI was added to the soil at the rate of 2.33 kilograms per acre. The soil in these ex-

periments was good and had good care. On Plot I the first planting was nearly mature at the time of application and was harvested within one week of the application of potassium iodide. Samples from this plot showed a very slight increase in iodine over the check.

On Plot II the second planting of lettuce was cut 15 days after the application. The plants in this plot were practically normal except for very slight stunting of their growth. The content of iodine was increased considerably over the check and Plot I.

On Plot III the third planting was cut 30 days after the application of potassium iodide. There was no evidence of either reduction or increase in the yield of this treated plot. The iodine content was slightly greater than in Plot II and was almost ten times as great as the content in the check.

TABLE 4
*Effect of Iodine Fertilizer on the Iodine Content
of Leaf Lettuce*

Plot	Treatment	Iodine ¹ — dry basis	Plot	Treatment	Iodine— dry basis
I	5 lbs. (KI per acre) applied 7 days before harvest	2,260	III	5 lbs. (KI per acre) applied 30 days be- fore harvest	13,450
		3,400			14,110
		1,510			13,140
II	5 lbs. (KI per acre) applied 15 days be- fore harvest	8,820	IV	44 lbs. (KI) applied 5 days before harvest	123,270
		9,280			118,310
		8,610			109,580
			Check		1,590
					2,050
					1,420

¹ All analyses calculated as parts per billion (p.p.b.) of the moisture-free sample.

On Plot IV another planting was treated at the time of harvesting Plot III. This plot received an application of 20 kilograms per acre of KI, nine times as great as the application of the other plots. The lettuce in this planting needed about one week to reach maturity. In three days the plants in this plot showed injury. The lettuce was stunted in growth, the veins discolored, and there were dead areas in the leaves. In a week all the plants in this plot showed severe injury. The leaves showed the dead veins, and several plants had collapsed. The check was normal. Analyses of samples from the treated area showed tremendous increase in iodine content. All leaf lettuce fertilizer plots were located in Clarence Salzgeber's greenhouses near Lane Avenue, Columbus, Ohio.

EXPERIMENTS WITH TOMATOES

Potassium iodide was added in very small quantities to eight plots of tomatoes replicated four times in the Ohio State University green-

houses. Each plot consisted of five plants separated by a guard of two plants. There was a guard row between each series of treatments.

Iodine was added to each of eight plots in the following concentrations:

Plot No.	Amt. per acre KI in grams	
1	250	
2	500	
3	750	
4	1,000	
5	1,000	—Sulfur
6	15,250	(First application of 250 gm., two later applications of 7,500 gm. each. First application only made prior to first analy- sis).
7	1,250	
8	1,500	
Check	None	

The iodine was added in the form of KI in a water solution and sprinkled evenly over each plot of five plants.

The results of the tomato work indicate a slight increase in the iodine content when almost any amount of KI was added to the soil. The soil in this experiment was very rich and had a pH of about 7.2. The KI was added when the plants were about three feet tall and

TABLE 5
*Results of Applications of KI on Iodine Content
of Tomatoes*

Plot No.	Iodine ¹ —dry basis				Av. 4 rep- lications— first test	Av. second test ²
	Rep. 1	Rep. 2	Rep. 3	Rep. 4		
1.....	231	252	231	252	267	241
2.....	252	273	273	294	273	231
3.....	294	273	252	273	273	252
4.....	252	315	252	273	273	252
5.....	252	231	231	252	242	210
6.....	210	252	252	231	236	431 ³
7.....	315	294	315	273	299	294
8.....	336	315	336	357	336	336
Check.....	210	231	250	231	231	210

¹ All analyses calculated as parts per billion (p.p.b.) of the moisture-free sample. ² Because of the smallness of samples second tests were made with samples from the four replications in one determination. ³ At the time of this analysis, Plot 6 had received the equivalent of 15.3 kg. of KI in three applications, one of 25 kg., and two of 7.5 kg. The heavy application did not noticeably affect the growth or yield of the tomatoes.

the first cluster was set and the second cluster was in full bloom (except as noted in Plot 6). Samples were analyzed five and six weeks after the treatment (Table 5).

ANALYSES OF ASPARAGUS

A sample of asparagus labeled "Asparagus from the Iodine State" was secured. This was compared with asparagus grown on the University Farm at Columbus (Table 6).

TABLE 6
Iodine Content of South Carolina and Ohio Asparagus

Asparagus	Iodine ¹ — dry basis
Ohio sample.....	1,260
South Carolina sample.....	3,780

¹ All analyses calculated as parts per billion (p.p.b.) of the moisture-free sample.

SUMMARY

The iodine content of leaf lettuce grown in greenhouses throughout Ohio varies greatly. Leaf lettuce grown at the Yoder Bros. Greenhouses in Barberton had almost double the iodine content of California head lettuce; the iodine content of the greenhouse lettuce exceeded in two other localities, equaled in three localities, and was less than that of California head lettuce in only two localities. The iodine content of leaf lettuce can be increased by the use of KI at the rate of five pounds per acre applied at least 15 days before harvest. An application of 44 pounds of KI per acre caused severe burning of leaf lettuce.

The iodine content of tomatoes can be increased slightly by the application of 30 pounds of KI per acre.

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STABILITY OF VITAMIN B₁ OF VACUUM-DRIED ANIMAL TISSUES DURING STORAGE ¹

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The stability of the antineuritic vitamin during storage has not been widely studied. Jones and Nelson (1930) noted a loss of vitamin B in tomatoes during storage. Douglass and Richardson (1930) detected no loss of the antineuritic factor during the storage of carrots in a humid cellar at 4.4°C. (40°F.) or in their laboratory where the air was dry. House, Nelson, and Haber (1930) reported that carrots showed no appreciable loss of vitamin B when stored in a storage cellar at 1.7 to 7.2°C. (35 to 45°F.) with humidity of 85 to 87 per cent for a period of four months. Langley, Richardson, and Andes (1933) found that the vitamin B content of carrots was not diminished by four months' storage in a cool, damp cellar or for a similar period in a warm, dry cellar. A loss of approximately 50 per cent of the vitamin B was noted by this last group of investigators in canned carrots after six months of cellar storage. The stability of the antineuritic factor in fruits and vegetables has been discussed by Fellers (1936).

In connection with another problem at this laboratory several vacuum-dried animal tissues were obtained in vacuum-packed cans.³ The tissues were assayed for their vitamin B₁ potency by the chick-assay method, according to Elvehjem (1935, 1936), soon after having been packed. Several tins of each product were stored for two years at room temperature and the antineuritic potency of the tissues was again determined.

EXPERIMENTAL PROCEDURE

Day-old white Leghorn chicks with an initial weight of about 35 grams were obtained from the University Poultry Department. The chicks were placed in cages equipped with suitable warmers and raised wire-screen floors (two meshes to the inch). Each group usually contained four chicks. Water was supplied in porcelain cups daily. The chicks were weighed weekly.

¹ Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

² Wilson and Company Fellow.

³ The animal tissues used in this study were supplied by Mr. L. M. Tolman of Wilson and Company, Chicago.

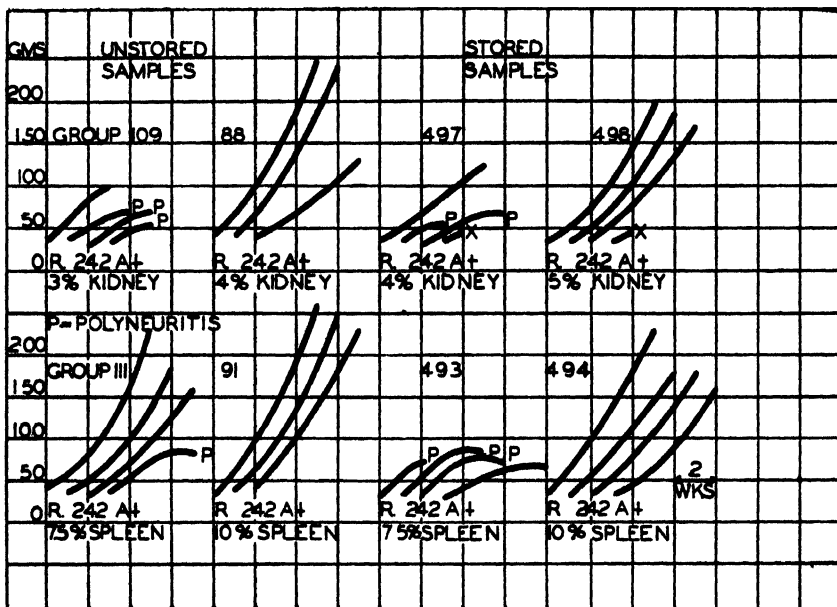


FIG. 1. Individual growth records of chicks fed Ration 242A supplemented with unstored and stored (two years) samples of vacuum desiccated, vacuum-packed beef kidney or beef spleen.

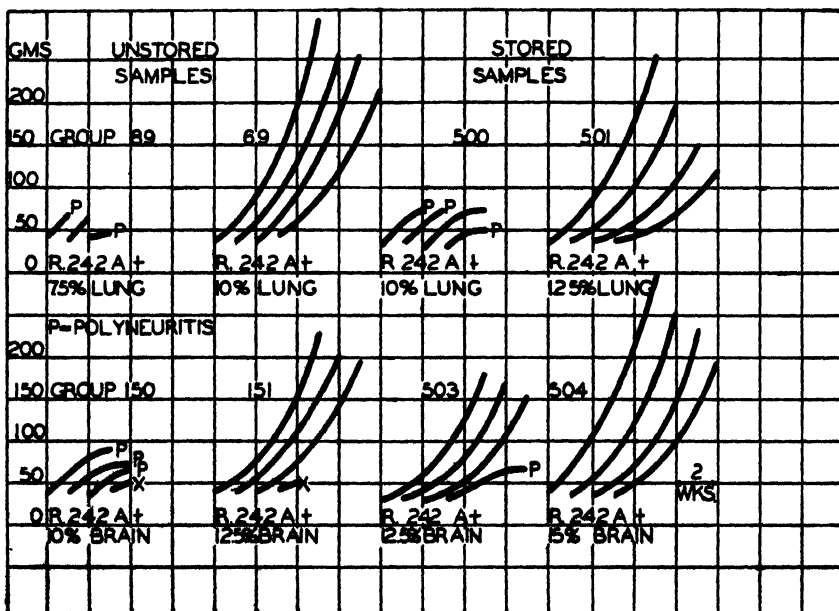


FIG. 2. Individual growth records of chicks fed Ration 242A supplemented with unstored and stored (two years) samples of vacuum-desiccated, vacuum-packed beef lungs or hog brains.

The basal vitamin B₁ low ration (Ration 242A) of Elvehjem (1935, 1936) was used throughout. It has the following composition:

Autoclaved portion:

Ground yellow corn.....	57
Pure flour middlings.....	25
Crude domestic acid precipitated casein.....	12

Untreated portion:

Vacuum-desiccated whole liver substance ⁴	2
Iodized salt (.02% potassium iodide).....	1
CaCO ₃ (precipitated).....	1
Ca ₃ (PO ₄) ₂ (precipitated).....	1
Cod liver oil.....	1

The animal tissues were tested for their antineuritic potency by substitution into the ration at various levels. In each case the smallest level of material necessary to protect all the chicks in the group from polyneuritis over a period of five weeks was determined. Any material fed on the percentage basis which protected the chicks for five weeks was found to continue to do so indefinitely. The rations were made up fresh weekly. In addition to the basal ration the chicks received two drops of haliver oil twice weekly to insure an adequate supply of vitamin A.

The vacuum-desiccated samples of kidney tissue (beef), spleen tissue (beef), lung tissue (beef), and brain tissue (hog) were obtained in two-pound vacuum-packed tins. The tissues were mixed with an equal weight of the autoclaved component of the ration and finely ground in a Burr mill before use. The tissues were then incorporated into the rations in the proper proportions.

RESULTS

The animal tissues used in this study were rich sources of the antineuritic factor.

Beef Kidney (Fig. 1). A small but definite decrease in antineuritic potency was apparent in the sample of kidney after a storage period of two years. The assay of the unstored sample of kidney for the antineuritic vitamin showed that the chicks were protected from polyneuritis when Ration 242A was supplemented with the vacuum-desiccated sample at a four-per cent level (Group 88). The stored sample of kidney was not effective in preventing the onset of polyneuritic symptoms in two of the chicks in Group 497 when fed at the same level in Ration 242A. Good growth and protection against polyneuritis resulted when the chicks were fed Ration 242A supplemented with five per cent of the stored sample of kidney (Group 498).

⁴ Wilson Laboratories, Chicago.

Beef Spleen (Fig. 1). There was little, if any, loss of vitamin B₁ in vacuum-dried, vacuum-packed spleen after a storage period of two years. Both samples protected the chicks from polyneuritis when fed at a 10-per cent level in Ration 242A (Groups 91 and 494). When the spleen supplement to Ration 242A was reduced to a 7.5-per cent level one chick in Group 111 (fresh sample) and three of four chicks in Group 493 (stored sample) were not protected from polyneuritis.

Beef-Lung Tissue (Fig. 2). A small but distinct loss of vitamin B₁ was apparent during the storage of vacuum-desiccated lungs. Excellent growth and prevention from polyneuritis resulted when the chicks were fed Ration 242A supplemented with 10 per cent of the unstored sample of lungs (Group 69). Three of four chicks fed Ra-

TABLE 1

Effect of a Two-Year Storage Period on the Vitamin B₁ Content of Vacuum-Desiccated Animal Tissues Contained in Vacuum-Packed Cans as Determined by the Prophylactic Chick-Assay Method

Tissue	Protective level		Vitamin B ₁ content per gram, dry basis		Loss
	Fresh sample	Stored sample	Fresh sample	Stored sample	
	<i>pct.</i>	<i>pct.</i>	<i>I.U.</i>	<i>I.U.</i>	<i>pct.</i>
Beef spleen.....	10.0	10.0	2.0	2.0	Slight
Hog brain.....	12.5	15.0	1.6	1.3	18
Beef kidney.....	4.0	5.0	5.0	4.0	20
Beef lung.....	10.0	12.5	2.0	1.6	20

tion 242A supplemented with 10 per cent of the stored sample of lungs (Group 500) developed polyneuritis. An increase of the same supplement to a 12.5-per cent level (Group 501) gave borderline protection as shown by the varied rates of growth of the chicks.

Hog Brain (Fig. 2). A slight loss of vitamin B₁ occurred during the storage of vacuum-desiccated brains. The unstored sample of brain was effective in preventing the onset of polyneuritis in chicks when added to Ration 242A at a 12.5-per cent level (Group 151). One of four chicks developed polyneuritis when they were fed Ration 242A supplemented with 12.5 per cent of the stored sample of brains (Group 503). Excellent growth and prevention from polyneuritis resulted when the chicks were fed Ration 242A supplemented with 15 per cent of the stored sample of brains (Group 504).

Since chicks require approximately 20 International Units of vitamin B₁ per 100 grams of Ration 242A for protection from polyneuritis, as shown by Arnold and Elvehjem (1938), the results may be expressed in International Units (Table 1).

DISCUSSION

The limited loss of the antineuritic factor contained in dry animal tissues owing to a two-year storage period is in agreement with the results of previous investigators. Some variation in the stability of the vitamin in different tissues was indicated. Both the unstored and stored samples of spleen protected all the chicks in each group from polyneuritis for the five-week period when the chicks were fed Ration 242A supplemented with 10 per cent of these samples. The decrease in antineuritic potency of brain was not great, since only one of the four chicks fed Ration 242A supplemented with 12.5 per cent of the stored sample, the level which had given protection with the unstored sample, developed the polyneuritic syndrome. A definite loss of antineuritic potency was apparent after the storage of kidney and lungs for the two-year period. Two of the chicks fed Ration 242A supplemented with four per cent of the stored sample of kidney, the level which had given protection with the unstored sample, developed polyneuritis; and growth of the only surviving chick was poor. Similarly, three of the four chicks fed Ration 242A supplemented with 10 per cent of the stored sample of lungs, the level which had given complete protection and excellent growth with the unstored sample, developed the polyneuritic syndrome during the third week on the ration.

Since recent investigations on the vitamin B complex have disclosed a number of new factors, earlier studies on the stability of the antineuritic vitamin during storage may have measured the stability of more than vitamin B₁ since this was not the only factor supplied by the materials examined. The chick-assay procedure depends on the prevention of polyneuritis in chicks, which afforded a rapid and dependable method for the determination of the antineuritic factor.

CONCLUSIONS

The animal tissues studied were rich sources of the antineuritic factor. Results obtained by the chick-assay procedure were as follows: beef kidney, 5.0 I.U. per gram; beef spleen, 2.0 I.U. per gram; beef lung, 2.0 I.U. per gram; and hog brains, 1.6 I.U. per gram (all values expressed in terms of vacuum-dried sample).

Some variation in the stability of the antineuritic vitamin contained in vacuum-packed beef kidney, beef spleen, hog brains, and beef lungs during a storage period of two years in vacuum-packed cans was observed. Very little loss of the vitamin resulted during the storage of vacuum-desiccated beef spleen. The decrease in antineuritic potency of vacuum-desiccated samples of hog brains, beef kid-

ney, and beef lungs was small but significant. The loss ranged to approximately 20 per cent of the original vitamin B₁ content.

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THE ANTIRACHITIC EFFECT OF SOME FOODS

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In all the diets proposed for the production of rickets in the rat, the absence of vitamin D and the calcium-phosphorus ratio and level seem to be the rachitogenic factors generally agreed upon. The early suggestion of Mellanby (1921, 1925) that certain foods, particularly certain cereals, contain an anticalcifying factor seems no longer to receive a great deal of support. Recently Mottram and Palmer (1937) have given direct evidence of the nonexistence of a rickets-promoting substance in cereals of the kind suggested by Mellanby. There is reason, however, for making a distinction between chemical level and biologically available level and, on this ground, Bruce and Callow (1934) have called attention to the fact that the inositol hexaphosphoric acid in cereals is poorly assimilated. This serves as a possible explanation for the variation in severity of rickets produced from time to time by rachitogenic diets, since the different forms of phosphorus may occur in varying proportions from time to time in the same cereal even though the total phosphorus content may be the same. Harris and Bunker (1935) were unable, however, to demonstrate that either the absolute or proportional amount of phytin phosphorus correlates with the severity of rickets obtained. According to the experiments of Mottram and Palmer hydrolysis of the phytin phosphorus in cereals does not affect the rachitic condition of the animals receiving them.

The Steenbock and Black (1925) rachitogenic diet No. 2965 and the procedure these authors outline still remains the standard method for the production of rickets in the rat and the determination of the vitamin D, or the antirachitic principle, in foods. Its weakness as generally applied to materials other than the most potent, such as cod liver oil and vitamin D preparations, is that additions to the rachitogenic diet have been small in order to avoid upsetting the calcium and phosphorus relationship and thus gave no answer to the antirachitic effect of the food in question or others like it if they constituted the entire diet. Recently (1934) the use of chicks in the method as discussed by Griem and associates (1935) has been receiving attention because it involves no dietary abnormality such as the high calcium-phosphorus ratio in diet No. 2965.

The use of the Steenbock and Black diet No. 2965 in the assay of foods for their vitamin D content has led some writers to the conclusion that most common foods contain no significant quantity of vitamin D in terms of man's requirement. It therefore seems appropriate to adapt this method to the more normal dietary conditions employed in the experiments reported herewith. If it is true that the rachitogenic factors are bound up in the calcium-phosphorus ratio and level, then as long as the calcium-phosphorus ratio and level is kept constant a gradient substitution of any or all of the other dietary constituents up to any percentage substitution should make it possible to estimate the antirachitic principle in the diet used for substitution. A preliminary report of such a procedure has been made by Kohman, Eddy, Sanborn, and White (1934). More extensive experiments are herewith presented. In the preliminary report referred to, it was shown that when the Steenbock and Black diet No. 2965 was replaced by varying amounts of a mixture consisting of one can each of roast beef, peas, carrots, spinach, and sweet potatoes to which sufficient calcium carbonate had been added to produce the calcium-phosphorus ratio of diet No. 2965, normal bone ash resulted when diet No. 2965 was half substituted and the percentage of bone ash was not increased by the addition of cod liver oil. In those experiments the various diets were fed for six weeks after the weaning period with and without cod liver oil, and the percentage of bone ash was used as the criterion of degree of calcification.

EXPERIMENTAL WORK

The results obtained when the substitution was made only for the 10-day healing period after the prescribed 20-day clearing period called for in the standard procedure are shown (Table 1). It is evident that there is a gradual improvement of the rachitic condition as more and more of the diet No. 2965 is replaced by the other foods. The effect on percentage of bone ash in this 10-day period is omitted because it cannot be expected to be marked. This is also evident when cod liver oil is added. As was noted in the preliminary report, and as will be evident in the data herewith presented, the substitution of diet No. 2965 by the various food mixtures used tended to enhance the growth which, other conditions being equal, tends to enhance the rachitic condition. From an examination of Experiment 2 (Table 1) it appears that the usual growth increment is not noticeable during the 10-day period. It so happened that there was no growth in the cases where cod liver oil was supplied. This growth increment should in all cases be taken into consideration in comparing the results produced by the various diets. The composition of the

diet used in the substitution for diet No. 2965 (Table 1) is given (Table 1-A).

TABLE 1
Line Test and Growth Increment From 10-Day Substitution After 20-Day Clearing on Diet No. 2965¹

EXPERIMENT 1				
	Growth increment	Line test— degree of healing		
	gm.			
Control diet No. 2965.....	3	—		
5% substitution.....	6	—		
15% substitution.....	11	—		
30% substitution.....	15	+ to ++		

EXPERIMENT 2				
	With C.L.O.	Without C.L.O.	With C.L.O.	Without C.L.O.
Control diet No. 2965.....	—
15% substitution.....	0	8	3+ to 4+	— to +
30% substitution.....	—1	6	4+	+ to 4+
50% substitution.....	—2	+2	4+	2+ to 4+

¹ All results average of six animals.

TABLE 1-A
Composition of Diet Used for Substitution in Table 1
(Ca: P = 5.5:1)

	Contents per can			
	Wet weight	Solids	Calcium	Phosphorus
	gm.	gm.	gm.	gm.
Roast beef.....	362	133.3	0.054	0.455
Peas.....	607	110.6	0.106	0.474
Carrots.....	599	44.8	0.147	0.156
Spinach.....	523	34.5	0.324	0.136
Sweet potatoes.....	881	294.4	0.212	0.401
Calcium carbonate.....	19.6	7.680
Total.....	592.2	8.523	1.622
Per cent of solids.....	1.44	0.274

In the following experiments different foods were used to make up the mixtures with which the Steenbock diet No. 2965 was substituted in the hope that some light would be thrown on the particular food that might be most responsible for the calcifying effect. In all the later experiments the animals were fed their respective diets for 42 days, beginning with animals approximately 25 days of age. Litter mates were distributed as evenly as possible by weight and by sex between the different diets in each experiment. The diet used in the

experiment (Table 2), in which for the sake of convenience one can of each of the individual foods was used to make up the mixture, is shown (Table 2-A). There is no reason for believing that the foods and diets involved in these experiments would perform differently in any other form.

In order to have a diet with a low phosphorus content the mixture, analysis of which is given (Table 2-A), was used. The means

TABLE 2

Line Test, Bone Ash, and Weight Gain of Animals on Respective Diets From 24 to 66 Days of Age¹

	Weight gain	Line test—degree of calcification	Bone ash		
			Av.	Min.	Max.
	<i>gm.</i>		<i>pct.</i>	<i>pct.</i>	<i>pct.</i>
Control diet No. 2965.....	12	—	30.6	28.2	33.4
25% substitution + O.O.	34	2+	48.5	46.6	50.2
25% substitution + C.L.O.	13	4+	52.9	50.8	55.6
50% substitution + O.O.	8	3+	57.8	55.8	59.3
50% substitution + C.L.O.	7	4+	57.4	54.4	60.5
100% substitution + O.O.	—3	4+	58.0	56.1	59.3
100% substitution + C.L.O.	—2	4+	57.7	55.9	59.4

¹ All results average of six animals. Average initial weight 39 grams.

TABLE 2-A

Composition of Diet Used for Substitution in Table 2

(Ca : P = 5.9 : 1)

	Contents per can				
	Wet weight	Solids	Calcium	Phosphorus	Nitrogen
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Chicken.....	344	105.0	0.045	0.485	14.79
Peas.....	597	104.0	0.113	0.442	4.29
Asparagus.....	572	38.0	0.080	0.275	2.00
Peaches (2 cans).....	1650	345.0	0.050	0.248	0.99
Calcium carbonate.....	20.7	8.272
Total.....	612.7	8.560	1.450	22.07
Per cent of solids.....	1.40	.237	3.27

by which the phosphorus content of the mixture was lowered in this diet was the large proportional use of canned peaches. Since peaches are canned in a rather heavy syrup, the phosphorus content was lowered in reality by dilution with the cane sugar used in canning. Approximately 60 per cent of this diet on a solids basis was supplied by the peaches, and two-thirds to three-fourths of the solids in the canned peaches consisted of cane sugar used in canning them. The diet represented in Table 2-A is, therefore, composed of only approxi-

mately 60 per cent natural food product on a solids basis, the other 40 per cent being cane sugar supplied by the peaches. This should be borne in mind in evaluating the calcifying effect as recorded (Table 2). It will be observed that the lowest substitution tended to enhance the growth increment but higher substitutions did not, and when the substitution was 100 per cent there was an actual loss in weight. Two reasons undoubtedly account for this: one, the highly liquid nature of the diet owing to the syrup on the peaches and the other, the high sugar content. In spite of these abnormalities, calcification was almost maximum even when there was the most rapid growth with the 25-per cent substitution, which is really only a 15-per cent substitution of natural food products. The addition of cod liver oil, in dosages more than enough to produce the maximum effect with the Steenbock and Black diet No. 2965, only slightly increased the percentage of bone ash. With the 50-per cent substitution—that is, a 30-per cent substitution of natural food product—cod liver oil does not cause a higher percentage of bone ash. Olive oil, equal to the amount of cod liver oil used, was added to the control diet.

In order to have a diet with a lower water content and to further lower the phosphorus content, starch was used as a diluent in a diet (Table 3-A). From the results on the animals (Table 3) it is apparent that with a 25-per cent substitution of the rachitogenic diet No. 2965 there was practically normal calcification. In this experiment all substitutions including complete substitution, resulted in better growth than the control diet which should make it a more severe test of the antirachitic principle.

Rickets in children is limited to the age of a complete or nearly complete milk diet. Milk may therefore be looked upon as containing little vitamin D. That being the case there should be little antirachitic or calcifying principle in casein. With this in mind, the diet in Table 4-A was prepared for an experiment conducted on the same basis as that represented by Table 3. This diet differs only in the use of casein and Mazola oil for the protein and fat respectively of the chicken. An amount of casein was used the nitrogen content of which exactly corresponded to that of the chicken and an amount of Mazola oil was used that corresponded with the ether extract of the dried chicken. The slight error that starch was regarded as without any moisture content (Table 3-A) is corrected (Table 4-A). The casein obtained from the Casein Manufacturing Company of America, Inc., supplied slightly more phosphorus (.006 per cent) than the chicken and this called for a little larger addition of calcium carbonate to secure the same calcium to phosphorus ratio. This

change in the percentage of calcium, phosphorus, and nitrogen is insignificant.

As is evident (Table 4), casein plus Mazola oil does not have the calcifying effect of chicken. With a 25-per cent substitution there is no apparent improvement in the percentage of bone ash. With a 50-per cent substitution, there is apparently a small but significant

TABLE 3

Line Test, Bone Ash, and Weight Gain of Animals on Respective Diets From 26 to 68 Days of Age¹

	Weight gain	Line test—degree of calcification	Bone ash		
			Av.	Min.	Max.
	gm.		pct.	pct.	pct.
Control diet No. 2965 + C.L.O.	10	4+	54.2	51.4	58.8
Control diet No. 2965 + O.O.	13	—	38.4	34.3	40.9
25% substitution + C.L.O.	24	4+	54.6	52.1	55.8
25% substitution + O.O.	36	4+	50.6	48.3	54.9
50% substitution + C.L.O.	28	4+	57.5	53.1	61.4
50% substitution + O.O.	36	4+	60.2	55.2	63.2
100% substitution + C.L.O.	15	4+	59.1	56.1	60.9
100% substitution + O.O.	17	4+	59.6	57.4	61.0

¹ All results average of six animals. Average initial weight 43 grams.

TABLE 3-A

Composition of Diet Used for Substitution in Table 3

(Ca : P = 5 : 3 : 1)

Contents per can

	Wet weight	Solids	Calcium	Phosphorus	Nitrogen
	gm.	gm.	gm.	gm.	gm.
Chicken.....	344	105.0	0.045	0.485	14.79
Peas.....	597	104.0	0.113	0.442	4.29
Peaches.....	825	173.0	0.025	0.124	0.50
Turnip greens.....	562	31.0	0.894	0.124	1.63
Starch.....	162	162.0
Calcium carbonate.....	12.7	5.080
Total.....	587.7	6.157	1.175	21.21
Per cent of solids.....	1.05	0.20	3.61

improvement in percentage of bone ash since statistical treatment of the data by Students Method indicates only one probability in a hundred that the improved result is mere chance. In this connection, it must be borne in mind that the peaches and peas (minus added sugar) and turnip greens supply a certain portion of the diet in the form of natural food products. In the diet in Table 4-A the solids in the peas, peaches, and turnip greens natural to those three foods,

constitute less than 30 per cent of the total solids. When a 50-per cent substitution is made, therefore, these three foods constitute less than 15 per cent of the total diet. A comparison of the data (Tables 3 and 4) therefore warrants the conclusion that whereas casein and Mazola oil exert no antirachitic or calcifying effect, chicken very markedly exerts such an effect. Furthermore, the peas, peaches, and

TABLE 4

Percentage of Bone Ash and Weight Gain of Animals on Respective Diets From 26 to 68 Days of Age¹

	Number of animals	Weight gain	Line test—degree of calcification	Bone ash		
				Av.	Min.	Max.
Control diet No. 2965.....	11	17	—	35.7	32.6	38.9
Control diet No. 2965 + M.O.	11	14	—	35.4	32.6	39.1
Control diet No. 2965 + C.L.O. ..	9	11	4+	53.2	51.4	55.1
25% substitution + M.O.	14	16	—	34.7	30.8	41.0
25% substitution + C.L.O.	12	25	4+	54.7	52.5	56.4
50% substitution + M.O.	14	33	+ to 2+	38.3	31.9	43.0
50% substitution + C.L.O.	14	40	4+	58.4	55.4	61.0

¹ Average initial weight 41 grams.

TABLE 4-A

Composition of Diet Used for Substitution in Table 4

(Ca : P = 5.3 : 1)

	Contents per can				
	Wet weight	Solids	Calcium	Phosphorus	Nitrogen
	gm.	gm.	gm.	gm.	gm.
Casein.....	102	96.00	0.010	0.529	14.79
Peas.....	597	104.00	0.113	0.442	4.29
Peaches.....	825	173.00	0.025	0.124	0.50
Turnip greens.....	562	31.00	0.894	0.124	1.63
Mazola oil.....	10.41
Starch.....	181	162.00
Calcium carbonate.....	13.31	5.324
Total.....	589.72	6.366	1.219	21.21
Per cent of solids.....	1.08	.206	3.60

turnip greens when constituting 15 per cent of the total diet on a solids basis slightly increase the percentage of bone ash.

Harris and Bunker (1937) recently reported on "The Anti-rachitic Property of Casein," stating they were unable to produce rickets in rats when casein furnished all the protein of the diet. Their results and those indicated (Table 4) appear to be inexplicably in disagreement except that the casein in our experiment did not fur-

nish all the protein but merely an amount comparable to that furnished by the chicken and roast beef. Scrutiny of Table 4-A reveals the fact that the 14.79 grams of nitrogen supplied by the casein in 589.72 grams of total solids is equivalent to 15.7 per cent of protein. This in itself should be adequate as a source of protein in the diet. Surely the remaining 6.42 grams of nitrogen supplied by the peas, peaches, and turnip greens and amounting to 6.8 per cent of additional protein in the diet could not be argued to be rachitogenic when imposed upon an already adequate protein supply furnished by the casein. Harris and Bunker's viewpoint regarding casein was not available at the time this experiment was begun.

Unfortunately, through a desire to use more animals with the smaller substitutions, the diet (Table 4-A) was not used to substitute more than 50 per cent of rachitogenic diet No. 2965. Whatever effect was produced by the 50-per cent substitution is logically to be ascribed to the somewhat less than 15 per cent of solids supplied by the peas, peaches, and turnip greens. If, according to Bruce and Callow, the rachitogenic properties of cereals is to be ascribed to the non-availability of a portion of the phosphorus; casein, whose phosphorus is presumably all available, should bring about improved calcification. The fact that it fails while chicken does improve calcification suggests that there are other factors operating than phosphorus availability.

SUMMARY AND CONCLUSIONS

1. In a series of experiments a low phosphorus level and the ratio of calcium to phosphorus of the Steenbock and Black rachitogenic diet No. 2965 were rigidly adhered to while substituting for it varying proportions of various food mixtures.

2. The substitutions were made in some experiments over the 10-day healing period after the usual 20-day clearing period on diet No. 2965, while in others they were made over a period of six weeks after weaning. The line test and percentage of bone ash were used as criteria of degree of calcification.

3. Fifty-per cent substitutions of several mixtures of natural foods produced maximum calcification that was not enhanced by the addition of cod liver oil even when the phosphorus content was only .2 per cent. Statistical treatment of the data when only 25- or 30-per cent substitutions were made indicate the addition of cod liver oil produced a slightly increased but significantly higher percentage of bone ash.

4. When casein and Mazola oil were used in place of the meat portion (canned roast beef or canned chicken) there was no evidence

of improved calcification from a 25-per cent substitution but small although significantly improved calcification with a 50-per cent substitution. This is logically ascribed to the approximate 15 per cent of vegetable and fruit solids in the mixture in which casein was used.

5. Since the phosphorus of casein is presumably as available as that in the meat used, the improvement in calcification caused by the meat cannot be explained on the basis of a less available form of cereal phosphorus in diet No. 2965 being replaced by a more available form in the meat.

6. The results therefore indicate a definite calcifying or anti-rachitic principle in the foods in question.

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A NOTE ON THE BIOASSAY TECHNIQUE FOR DETERMINING AVAILABLE IRON IN FOODS¹

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Smith and Otis (1936, 1937a) have reported difficulty in employing the Elvehjem, Hart, and Sherman (1933) procedure for the measurement of available iron in foods of low iron content because the experimental animals were unable to consume sufficient test material to obtain .3 mg. of available iron daily. In this laboratory we have also experienced this difficulty when analyzing various foods for hematopoietic potency if they contained less than .00160 per cent total iron. Since all of the common fruits and most vegetables are included in this category, as noted by Stiebling (1932), it seemed important to modify the method to include these foods.

An attempt was made, by supplementing a constant amount of test material with various quantities of iron as ferric chloride, to determine the iron necessary for hemoglobin regeneration equal to that induced by .3 mg. of iron as ferric chloride. The difference between .3 mg. and the quantity of iron found to be necessary could be considered the available iron content of the food under test.

The data presented (Table 1) indicate that this modification while retaining a principal feature of the original method, i.e., comparison of response at a .3 mg. daily level of iron feeding, is impractical for the following reason. The difference in hemoglobin increase over the six-week test period owing to .2 mg. of available iron was only slightly less than that caused by .3 mg. of available iron. Consequently, in feeding .1 mg. of iron as food-iron plus .20, .225, .25, and .275 mg. of iron as ferric chloride, the experimental and animal variations resulted in hemoglobin changes greater than those induced by the available portion of the food-iron. Statistically, there was no real difference between the hemoglobin response of Groups 1, 2, 3, 4, 5, and 6 of the animals. Therefore, no estimate of percentage availability was possible.

A further attempt to determine small quantities of available iron resulted in a procedure similar to that proposed by Smith and Otis (1937a). Data are presented (Table 2) from which a curve, stand-

¹ Presented in part before the South Carolina Academy of Sciences, April, 1938.

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ard for this laboratory, was constructed (Fig. 1) showing the relation between available iron intake and hemoglobin increase. The range of iron intake studied was from .01 to .30 mg. daily and the

TABLE 1
Comparative Hemoglobin Regeneration in Rats Fed a Constant Level of Food Iron Plus Varying Amounts of Inorganic Iron

Group No.	Iron supplement fed daily	Total iron in supplement	Number of animals used	Hemoglobin	
				Initial	Increase after 6-week experimental period
		mg.		gm./100 c.c.	gm./100 c.c.
1	.2 mg. Fe as FeCl ₃	.200	10	3.16	9.43 ± 0.18
2	.2 mg. Fe as FeCl ₃ + 16.67 gm. banana pulp	.300	8	3.32	10.11 ± 0.30
3	.225 mg. Fe as FeCl ₃ + 16.67 gm. banana pulp	.325	8	3.29	9.64 ± 0.26
4	.250 mg. Fe as FeCl ₃ + 16.67 gm. banana pulp	.350	8	3.40	10.21 ± 0.29
5	.275 mg. Fe as FeCl ₃ + 16.67 gm. banana pulp	.375	8	3.18	9.60 ± 0.20
6	.3 mg. Fe as FeCl ₃	.300	10	3.30	10.04 ± 0.14

length of the experimental period was six weeks. We confirmed the findings of Smith and Otis (1937a) that hemoglobin increase at different levels of iron feeding below .3 mg. was as uniform at the end

TABLE 2
Hemoglobin Regeneration in Anemic Rats

Group No.	Amount of Fe as FeCl ₃ in daily supplement	Number of animals used	Initial hemoglobin	Gain in hemoglobin during 6-week period
	mg.		gm./100 c.c.	gm.
1 and 8	.30	15	3.25	10.16 ± .17
6 and 7	.20	15	3.31	9.30 ± .21
9	.17	8	3.25	7.93 ± .26
10	.13	7	3.62	6.50 ± .15
11	.10	8	3.20	4.80 ± .21
12	.07	7	3.46	3.77 ± .26
13	.03	8	3.29	1.62 ± .27
14	.01	10	3.33	0.43 ± .30

of a four-week test period as at the end of six weeks but that some sensitivity would be sacrificed by terminating the experiment before the end of the six-week period.

Cognizance was taken of the fact that comparisons of iron availability should be confined to members of the same sex, as noted by

Mitchell and Hamilton (1937) and Smith and Otis (1937b), and only male rats were used in these experiments.

The animals were made anemic according to the method of Elvehjem and Kemmerer (1931) except that whole milk powder (Klim) was substituted for fresh liquid milk. When the hemoglobin of the rats reached a concentration between three and four grams per 100 ml. of blood, the animals were placed on experiment. We found that to continue the depletion to a hemoglobin level of two

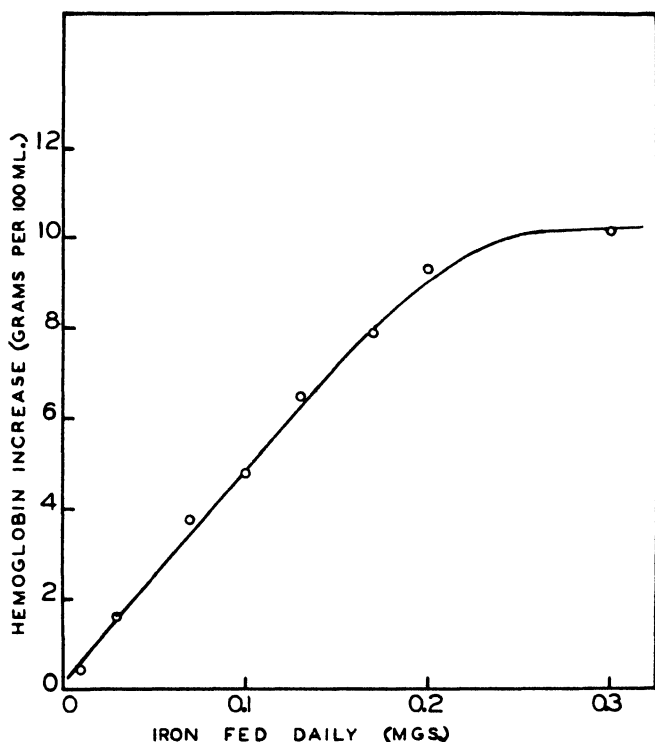


FIG. 1. Hemoglobin increase of male rats at various levels of iron intake over a six-week period.

to three grams, as recommended by Elvehjem and Kemmerer (1931) and Sherman, Elvehjem, and Hart (1934), exhausted the rats to a point where the oral administration of iron resulted in no hemoglobin response or at most a very erratic and uncorrelated response. It is clear that severely depleted rats respond less uniformly in hemoglobin increase and in weight gain to iron feeding than do rats more moderately depleted (Table 3). Smith and Otis (1937a) placed their animals on experiment when an average hemoglobin of 3.9 or 4 grams was reached, which corresponds closely with the practice in this lab-

TABLE 3
Comparative Uniformity of Hemoglobin Regeneration in Severely and in Moderately Anemic Rats

Group No.	Number of rats used	Iron supplement fed daily	Hemoglobin (gm./100 c.c.)							Coefficient of variation
			Initial		Final		Increase after 6 weeks			
			Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Probable error	
11	8	.10	gm. 3.20	gm. .38	gm. 8.00	gm. 0.57	gm. 4.80	gm. 0.81	± .21 16.9	
15	8	.10	gm. 2.11	gm. .46	gm. 6.14	gm. 1.69	gm. 4.03	gm. 1.70	± .51 42.2	

oratory. Hemoglobin determinations by the Newcomber acid-hematin method and erythrocyte counts were made at weekly intervals. Since changes in number of red blood cells paralleled changes in hemoglobin content in all of the animals studied, no use was made of the erythrocyte values in the interpretation of data.

No studies have been carried out in this laboratory to show the effect of heat upon the availability of food-iron but it has been noted that several references to this question occur in the literature. Shackleton and McCance (1936) observed that the cooking and canning of peas, beans, and meat apparently increased the availability of their iron content. Sherman, Elvehjem, and Hart (1934) found, however, that the roasting of soy beans slightly decreased the availability of some of the food-iron. It would seem logical to assay foods for available iron when they are prepared as for human consumption.

SUMMARY

We are in complete agreement with certain recommendations of Smith and Otis (1937a) for the modification of the Elvehjem bioassay procedure for determining available iron. Our data substantiated their practice (1) of constructing a standard curve of reference showing average hemoglobin increase as a function of available iron intake for levels between .01 and .3 mg. of iron daily, (2) of feeding the test material at a level conveniently consumed and to contain, if possible, less than .2 mg. available iron daily, (3) of comparing the average hemoglobin increase induced by the food under test with the reference curve and reading the available iron content of the food directly from the curve, (4) of advising the use of a six-week test period for extreme accuracy of results, and (5) of making comparisons of iron availability only between animals of the same sex.

In addition, it seemed from our results that rats whose hemoglobin had reached the level of three to four grams per 100 ml. of blood should be considered sufficiently depleted of iron storage and placed on experiment. Further depletion decreased the uniformity and accuracy of their response to subsequent iron feeding. Also, the foods to be tested should be prepared for bioassay exactly as they would be prepared for human consumption. If the food is always eaten raw it should not be cooked or dehydrated before feeding to the anemic rats regardless of convenience. Foods that are normally cooked for human consumption should not be assayed in the raw state since there is some evidence that heating may in some cases decrease and in other instances increase the availability of food-iron.

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BACTERICIDAL ACTIVITY OF CROTONALDEHYDE

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Walker, Lindegren, and Bachmann (1925); Walton, Herbold, and Lindegren (1936); and McKnight and Lindegren (1936) showed that the volatile substances from freshly crushed garlic and from onions are bactericidal. Vollrath, Walton, and Lindegren (1937) provisionally identified acrolein or crotonaldehyde as the bactericides.

EXPERIMENTAL PROCEDURE

Two series of aqueous solutions of crotonaldehyde were prepared in various concentrations. To one series 10 per cent by volume of sterile natural white of egg containing about 13 per cent of protein was added. To each tube holding 10 c.c. of solution, .1 c.c. of a heavy suspension of an 18-hour culture of *Escherichia coli* was added. At various time intervals (Table 1) streaks were made on agar plates. After incubation the plates were scored. The number "4" indicates growth equivalent to that of the respective control; "0" indicates no growth. The figures "1", "2", and "3", show intermediate degrees of growth. This experiment was duplicated with acrolein. Egg white inhibited the bactericidal effects of acrolein to some extent, but crotonaldehyde was only slightly inhibited, indicating that it is the better antiseptic.

The bactericidal effects of the vapors were tested by placing one c.c. of various dilutions on a sterile piece of filter paper in the top of an inverted petri dish and exposing the agar in the bottom of the dish to the rising vapors for one hour, after which the tops were exchanged for dry covers. Streaks were made on each plate with a heavy suspension of an 18-hour culture of *Escherichia coli* (Table 2). Crotonaldehyde vapors from aqueous solutions poison the agar in a manner similar to the vapors from crushed garlic. Egg white in the solution does not materially reduce the escape of vapor.

Dilutions of crotonaldehyde, acrolein, and formaldehyde from 1-100 to 1-10,000,000 were made up in nutrient agar and the tubes streaked with *Escherichia coli*. In each of the three series, growth was inhibited or prevented in the 1-10,000 dilutions, indicating that

TABLE 1
Bactericidal Effects of Acrolein and Crotonaldehyde Solutions

[illegible]

when an agar plate is exposed to the vapors of garlic, onions, or the pure aldehydes, the concentration at the surface of the agar reaches at least 1-10,000. This concentration, however, is localized at the surface and when a plate, poisoned by exposure to the vapors, is allowed to stand for a few hours before streaking bacteria on the surface the aldehydes diffuse through the agar and the concentration is too low to prevent growth.

DISCUSSION

Vapors from about one-per cent solutions of either acrolein or crotonaldehyde will poison the surface of agar plates. This suggests that these compounds are present in onions and garlic in concentrations of about one per cent. The odor and the irritating effect on the eyes of the vapor from the cut surface of an onion suggests

TABLE 2
Bactericidal Effects of Acrolein and Crotonaldehyde Vapors

Acrolein				
Dilutions.....	1/20	1/40	1/80	1/160
Egg white absent.....	0	0	0	4
Egg white present.....	4	4	4	4
Crotonaldehyde				
Dilutions.....	1/160	1/320	1/640	
Egg white absent.....	0	1	4	
Egg white present.....	0	4	4	

acrolein, when allowance is made for the odor of sulphides. The pungent taste of a crushed clove of garlic suggests the presence of crotonaldehyde. The characteristic sulphides are bland in both cases. In this laboratory Miss Lucile Walton made the interesting observation that although freshly cut garlic gives off poisonous vapors, garlic allowed to stand in water in a closed container for 24 hours loses all bactericidal properties. This suggests that the denatured proteins differ from the natural proteins in their ability to react with aldehydes. Furthermore, when acrolein is added to the dead crushed garlic it soon loses its poisonous effects, probably because it combines with the denatured proteins.

When the vapors from freshly crushed onions and garlic are allowed to come in contact with filter paper moistened with piperidine and an aqueous solution of sodium nitro prusside, the characteristic color test of unsaturated aldehydes is obtained. But distillation by heating crushed garlic or onion does not yield a distillate which

will give the characteristic test, probably because the proteins have combined with the aldehydes before they can distill off.

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ACID-BASE BALANCE OF CEREALS AND SOME RELATED FOOD MATERIALS ¹

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INTRODUCTION

The role of the acid-base balance of the ash constituents of foods in animal and human nutrition is not fully determined. Berg (1925) concludes from the work of Salkowski (1871) that wholesome human food must contain more base than acid equivalents. Physicians often prescribe diets with a base balance. In calculous diseases of the urinary tract an acid ash or alkaline ash diet is recommended, depending upon the nature of the calculi, as noted by Clark (1936). Various claims have been made in the literature on the effect of the acid or base balance of the diet on the formation of dental caries by Kugelmas and King (1933), and on calcium metabolism by Davis (1935).

Unfortunately experiments with well-established uncontradicted results are wanting, possibly because of the laborious and time-consuming calculation method previously used for the determination of the acid-base balance of foods. A simple and more direct method has been developed in this laboratory which yields results quite comparable with those obtained by the old calculation method used by Davidson and LeClerc (1935). The new method also has the advantage that it furnishes values for the total alkalinity or acidity of the ash in addition to the acid-base balance values of the food materials, as it is possible that in future studies of nutrition certain results may be correlated directly with this factor.

In this work the new method was applied to determination of the acid-base balance of the more common cereals and of some related food materials. In view of the limited scope of the work the values reported here should not be considered as definitely established; it has been demonstrated by Davidson and LeClerc (1936) that the chemical composition of plant-food materials must be studied with the object of obtaining the range of variation rather than absolute values. It is believed, however, that in view of the fact that the cereals have certain characteristics in common as a group, the reported results give the true general trend of their acid-base balance values.

¹ Food Research Contribution No. 350.

In conclusion it may be stated that the object of this work was not to emphasize in any way the importance of the acid-base balance of foods in nutrition but merely to report and discuss certain chemical values obtained by analysis.

EXPERIMENTAL WORK

Cereals of known origin obtained from the Bureau of Plant Industry, U. S. Department of Agriculture, included two varieties each of corn, wheat, oats, and barley and one variety each of rye, rice, and millet. By way of comparison, buckwheat, which is not a cereal but is used as one, soybeans, jack beans, and potatoes were also analyzed.

The cereal products analyzed included wheat bran, wheat germ, white wheat flour and a standard loaf of white flour bread, brown and uncoated rice, pearl barley, buckwheat groats, and buckwheat flour. The brown rice was prepared in the laboratory from the rough rice, the bread was baked in the baking laboratory of this division, the other cereal products were standard materials purchased on the open market. The soybeans were obtained from the Bureau of Plant Industry and the jack beans from the South Carolina Agricultural Experiment Station. The potatoes, according to the market record, were of the Irish Cobbler variety grown in Lancaster, Pennsylvania. They were analyzed unpeeled and peeled.

For the determination of the acid-base balance the method developed in this laboratory by Davidson and LeClerc (1935) was used. The procedure was briefly as follows: Owing to the difficulty with which cereals are ashed the samples were limited to one gram of substance. The ashing was carried out as recommended by Sullivan and Near (1927); the materials were weighed out into platinum dishes, ashed in an electric muffle first at 400°C. (752°F.) and then allowed to remain in the muffle at 620°C. (1148°F.) overnight. The ash was dissolved in five c.c. of half-normal hydrochloric acid, filtered, thoroughly washed, and titrated back with tenth-normal sodium hydroxide, using phenol red as an indicator. One c.c. of the indicator prepared according to Clark (1928) was added to each solution (about 100 c.c. in volume) and the tenth-normal sodium hydroxide solution was added till the first change in color. The addition of the standard sodium hydroxide solution was then cautiously continued, drop by drop, pouring at intervals 10 c.c. of the titrated solution into a test tube, adding each time to the test tube two to three drops more of the phenol red indicator and matching it against a LaMotte phenol red pH 7.0 tube. The solutions were reserved for phosphorus determinations. The titration values were

corrected for losses of sulphur and chlorine occurring during unprotected combustion as previously described by Davidson and LeClere (1935).

The other analyses were made according to the methods of the Association of Official Agricultural Chemists (1930): phosphorus and chlorine volumetrically; sulphur gravimetrically, the materials having been ashed with magnesium nitrate.

RESULTS

The titration values of the ash and the acid-base balance values in cubic centimeters of normal acid or alkali per 100 grams of substance and also the percentages of total ash, sulphur, chlorine, and phosphoric acid are given (Table 1, Columns 1 to 6).

The ash of all cereals, with the exception of yellow corn, is slightly alkaline. The slight acidity of the ash of yellow corn will have to be corroborated by additional tests on other samples before any significance can be attached to it. Next to the cereals in alkalinity comes buckwheat. It has a higher alkalinity than the cereals but is close to them, justifying in this respect its popular classification as a cereal. Next come the soybeans, jack beans, and potatoes, all of which are distinctly higher in the alkalinity of the ash than the cereals.

While the term ash alkalinity frequently applies only to the alkalinity caused by carbonates and oxides, according to Tillmans and Bohrman (1921), in the study of the acid-base balance of food materials it signifies the total neutralizing value of the ash including that of the ash phosphates. Moreover, in the ash of cereals, carbonates and oxides are totally absent; and in view of the fact that their ash, with the exception of silica which is a chemically inert substance, is very largely composed of phosphorus and potassium, the explanation for the alkalinity of the ash in cereals is to be found in the quantitative relationship of these two elements. The presence of potassium and phosphorus in the stoichiometric relationship of monopotassium phosphate would impart to the ash an acid reaction; their presence in the relationship of dipotassium phosphate or tripotassium phosphate would result in higher alkaline titration values than those recorded here. The relation, therefore, between potassium and phosphorus in the ash of cereals is probably that of a mixture of monopotassium, dipotassium, and tripotassium phosphate.

The relatively high alkalinity of the bran-ash is due to its high phosphate content in which the stoichiometric relation of phosphorus to potassium is approximately the same as in the ash of whole wheat. The relatively high alkalinity of the bread-ash is undoubtedly due to the loss of chlorine from the sodium chloride which is added to

TABLE 1

Acidity and Alkalinity of Ash, Acid-Base Balance, Total Ash, Phosphorus, Sulphur, and Chlorine in Cereals and Some Related Food Materials; Relation of Alkalinity of Ash and Acid-Base Balance to Recovery of Sulphur, Chlorine, and Phosphorus From Unprotected Ash.¹

Material and variety	1 Acidity or alkalinity of ash ²	2 Acid base balance ²	3 Ash	4 P ₂ O ₅	5 S	6 Cl.	7 Alkalinity of ash on basis of % P ₂ O ₅ ³	Percentage recovery from unprotected ash ²		
								S	Cl.	P ₂ O ₅ ash dissolved in n/2 HCl
Corn, yellow, King.....	-3.27	-10.20	1.42	pct. 0.84	pct. .091	pct. .039	-3.89	pct. None	pct. None	pct. 10
Corn, white, Pride of Saline.....	+2.17	-5.42	1.37	0.77	.096	.058	+2.81	None	None	7
Oats, Victory.....	+4.30	-4.52	3.74	0.96	.139	.080	+4.48	24.7	None	26
Oats, Jeanette.....	+2.15	-9.66	3.55	1.11	.200	.068	+1.94	20.9	None	19
Oatmeal.....	+1.63	-10.40	1.98	1.11	.173	.046	+1.47	0.4	None	20
Barley, 2-row, Horn.....	+6.54	-4.80	2.42	0.75	.116	.189	+8.72	18.8	None	54
Barley, 6-row, Trebil.....	+2.18	-7.42	2.70	0.97	.095	.178	+2.25	22.8	None	20
Barley, pearl.....	+2.22	-3.67	0.64	0.34	.058	.088	+6.53	None	None	13
Wheat, Marquis.....	+1.10	-8.91	1.91	1.00	.150	.031	+1.10	1.9	None	7
Wheat, Federation.....	+1.65	-6.93	1.78	0.88	.111	.070	+1.87	5.8	None	27
Wheat flour.....	+0.55	-8.66	0.44	0.27	.115	.071	+2.04	None	None	33
Wheat bran.....	+8.71	-2.61	7.65	4.09	.184	.039	+2.13	10.3	None	13
Wheat germ.....	+4.30	-9.14	3.77	2.06	.186	.072	+2.09	2.5	None	22
Rice, rough, Blue Rose.....	+7.64	-5.57	2.02	0.31	.121	1.258	+24.64	22.1	79.5	100
Rice, brown.....	+0.55	-5.41	5.98	0.82	.078	.047	+0.67	5.3	None	11
Rice, uncoated.....	+3.29	-2.08	1.51	0.88	.074	.027	+3.72	None	None	14
Eye, Rosen.....	+1.14	-4.22	0.39	0.26	.075	.024	+4.38	None	None	11
Millet, Yellow Manitoba.....	+2.18	-7.31	3.37	0.91	.138	.037	+2.40	2.9	None	12
Rye, Rosen.....	+4.42	-4.42	2.35	1.07	.127	.051	+4.13	6.7	None	17
Buckwheat.....	+9.87	+3.84	1.95	0.76	.114	.019	+12.99	23.3	None	80
Buckwheat groats.....	+7.73	-2.10	1.98	1.00	.155	.016	+7.73	3.7	None	34
Buckwheat flour.....	+11.00	+1.32	2.35	1.09	.153	.031	+10.09	7.5	None	44
Soybean, Mammoth Yellow.....	+41.30	+28.7	4.26	1.21	.276	.019	+34.13	27.4	80.2	103
Soybean, Dixie.....	+42.50	+27.4	5.82	1.33	.305	.0074	+37.78	20.6	100.0	103
Soybean, Manchu.....	+35.10	+19.5	5.37	1.59	.318	.0074	+22.08	21.2	100.0	102
Jack beans.....	+23.00	+18.3	3.30	0.97	.177	.070	+23.71	57.1	100.0	100
Potatoes, unpeeled.....	+33.80	+28.9	4.78	0.72	.181	.408	+46.94	60.5	96.4	103
Potatoes, peeled.....	+31.90	+26.9	4.37	0.72	.172	.347	+44.31	57.3	95.7	101

¹ All results are expressed on moisture free basis. ² For sulphur and phosphorus, ash obtained without magnesium nitrate; for chlorine, without sodium carbonate. ³ Cubic centimeters of normal acid (—) or alkali (+) per 100 grams of substance

standard bread. Loaves of bread baked in the same way as the standard loaf but without salt and without salt and sugar, respectively, were similar in alkalinity to the ash of the flour from which the bread was baked.

The corrected balance of the cereals is acid in every case. This acidity is due entirely to their sulphur and chlorine content. The acid equivalent of the lost sulphur and chlorine is more than enough to offset the alkalinity of the ash. Buckwheat is again on the border line. The soybeans, jack beans, and potatoes have a distinct base balance after the corrections for lost sulphur and chlorine.

A study of results (Table 1) shows that the acid balances of the cereals are not closely correlated either with the alkalinity of the ash or with the percentages of phosphorus, sulphur, and chlorine. While all chlorine is lost from cereals during combustion, its content is relatively small and, owing to the fact that hydrochloric acid is monovalent, its acid equivalent is relatively small. The sulphur content is generally larger than that of chlorine and its acid equivalent is also higher, but sulphur is not all lost during combustion and the extent of its loss is variable. The variability of the factors in different directions and their intercancelling character precludes their close correlation with the acid-base balance.

The alkalinity of the ash calculated on the basis of one per cent phosphoric acid, the percentage recovery of sulphur and chlorine from the ash of the materials obtained from unprotected combustion, and also the percentage recovery of phosphoric acid when the unprotected ash was dissolved in five c.c. of half-normal hydrochloric acid can be seen (Table 1, Columns 7 to 10).

The incomplete recovery of sulphur and chlorine from the unprotected ash on one hand and of phosphorus on the other, is due to entirely different causes. The unrecovered sulphur and chlorine are actually lost when the material is not protected during combustion with magnesium nitrate and sodium carbonate respectively; the unrecovered phosphorus, however, is not lost but only converted into a form which is not precipitated with ammonium molybdate or magnesia mixture.

When the losses during unprotected combustion, apparent or real, are correlated with the alkalinity of the ash or with the acid-base balance, it is seen that the sulphur losses, while there is a great variation in them, are not related to either of the two factors. The losses of sulphur seem to depend primarily upon the nature of the sulphur compound present in the plant material, i.e., whether its sulphur is oxidized to sulphuric acid without the aid of oxidizing agents or is driven off before reaching that stage.

The losses of chlorine seem to be correlated with the acid-base balance; when there is not enough base in the plant the chlorine is driven off. While there was practically no chlorine lost from the soy-beans, jack beans, and potatoes, it was completely lost from all the cereals and cereal products when they were ashed without sodium carbonate (Table 1, Column 9). Bread with added sodium chloride is seemingly an exception, having retained about 80 per cent of its chlorine; but on the basis of absolute quantity more chlorine was lost from the bread than from all the other cereals and cereal products.

TABLE 2

Effect of Whole Wheat and Some of Its Individual Components on Loss of Chlorine From 10 c.c. of a Tenth-Normal Sodium Chloride Solution During Unprotected Combustion at 550-620°C. (1023-1148°F.)

Substances with which sodium chloride was ignited	Quantity used	n/10 chlorine found on ignition	n/10 chlorine lost
	gm.	c.c.	c.c.
Wheat.....	10.0	2.3	7.7
Cystine.....	0.5	9.6	0.4
Gum gluten.....	2.0	7.3	2.7
Starch.....	10.0	5.2	4.8
Dextrose ¹	2.0	9.8	0.2
	c.c.		
Monopotassium phosphate, tenth-molecular solution.....	10.0	4.0	6.0
Dipotassium phosphate, tenth-molecular solution.....	10.0	10.0	None
Monopotassium phosphate, tenth-molecular solution.....	10.0	10.0	None
	gm.		
Sodium carbonate.....	1.0

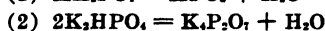
¹ Tenth-normal potassium chloride was used.

The relatively large absolute loss of chlorine (which comes largely from the added sodium chloride) from bread during unprotected combustion indicates that not all the chlorine lost from the cereals is necessarily organic in character.

The losses of chlorine from 10 c.c. of tenth-normal sodium chloride during unprotected combustion with whole wheat and some of its components are shown (Table 2). The losses of chlorine from the cereals during unprotected combustion are evidently caused jointly by the monopotassium phosphate, proteins, and starch. Dipotassium phosphate does not cause any loss of chlorine from sodium chloride. Sodium carbonate protects against loss of chlorine in all these cases.

The incomplete recovery of phosphorus is in a general way inversely correlated with the alkalinity of the ash. The cause of this incomplete recovery lies in the fact that the quantitative relationship

of potassium and phosphorus is largely that of a mixture of monopotassium and dipotassium phosphate. These, on heating at ashing temperature, form potassium metaphosphate and pyrophosphate respectively, according to the following equations:



Neither of these is precipitated with ammonium molybdate or magnesium mixture.

The values for the recovered phosphoric acid are not constant as they depend upon conditions of precipitation. The recovered phosphorus is made up of the orthophosphates originally present in the ash and those which originate from the hydrolysis of metaphosphates and pyrophosphates. In this work the phosphorus was precipitated with ammonium molybdate in a water bath at 49°C. (120.2°F.) in which the solutions were allowed to remain from 45 to 50 minutes—conditions favorable to the hydrolysis of metaphosphate and pyrophosphate. When determinations of recovered phosphorus were repeated by precipitating the ammonium phosphomolybdate in a shaking machine in the cold, some of the products gave appreciably lower values. All recorded determinations of recovered phosphoric acid, however, were made under approximately the same conditions and are, therefore, in a general way comparable.

The magnitude of the alkalinity values of the ash in the cereals depends upon two factors: the quality of the potassium phosphates (i.e., the quantitative relationship between potassium and phosphorus) and their absolute quantity. Just as 200 grams would give twice as large a titration value as 100 grams of the same material, so an ash containing twice as much potassium phosphate of the same composition as another would also give a titration value twice as high as the other for the same weights of the respective substances. In view of the fact that the percentage recovery of phosphorus from an unprotected ash would theoretically depend upon the composition of the potassium phosphates and not upon their quantities, it was thought advisable to recalculate all the alkalinity values to the basis of one per cent phosphoric acid. This was accomplished by dividing the alkalinity values by the corresponding percentages of phosphoric acid.

It is seen that the recalculated alkalinity values (Table 1, Column 7) are more closely correlated with the recovery of phosphoric acid than are the original values. It particularly explains why wheat bran with a higher original alkalinity value than bread gave a low

recovery while the latter gave a complete recovery of phosphorus. In a general way there is a rise in recovery of phosphorus with the rise in the recalculated alkalinity values. The recovery of phosphorus (orthophosphate content) in the ash of cereals and their products, with the exception of the bread ash, fluctuates between 7 and 54 per cent of the total phosphorus, barley (two-row), which has the highest alkalinity value of the cereals tested, giving the highest recovery. Buckwheat with an alkalinity value of 12.99 gave 80 per cent recovery, and complete recovery began at the alkalinity value of 22.08. Complete recovery of the phosphorus seems to lie between these two alkalinity values.

DISCUSSION

It has been shown that there is a distinct correlation between the alkalinity of the ash and of the acid-base balance of cereals and related food materials on the one hand and the results obtained from their combustion at ashing temperature on the other. The materials which gave an acid balance or only a slight base balance, as in the case of buckwheat, lost their entire chlorine content during unprotected combustion; the other materials which had a relatively large base balance retained practically all their chlorine. The transformation of the phosphorus into metaphosphate and pyrophosphate during combustion is clearly correlated with the low alkalinity of the ash.

These correlations may prove significant. Combustion of food at body temperature has a good deal in common with combustion at ashing temperature; carbon and hydrogen are oxidized to carbon dioxide and water and are eliminated during respiration, while the mineral elements are left behind. Sulphur and chlorine, as far as known, are not given off during respiration; however, it remains to be determined whether the sulphur and chlorine of the plant foods which are driven off during combustion at ashing temperature behave in metabolism as the sulphur and chlorine which are retained in the ash.

Of particular interest is the question whether the phosphorus which is converted into metaphosphate and pyrophosphate during ashing will be converted into the same forms during metabolism. Metaphosphate and pyrophosphate are known to be present in animal tissues and blood, as noted by Eggleton and Eggleton (1930) and Hawk and Bergeim (1931), and it is possible that they are derived from foods with a relatively low alkalinity of the ash.

It is possible, therefore, that the significance of the acid-base balance of the diet, besides its possible direct contribution to the acidity or basicity of the human and animal system, lies also in its effect on the nature of the products of mineral metabolism.

SUMMARY

The method developed in this laboratory for determining the acid-base balance of foods has been applied to cereals and some of their products and to some related food materials. It has been found that:

1. All cereals tested, with the exception of yellow corn, have a slightly alkaline ash; all, however, have an acid balance.

2. Buckwheat, which is commonly used as a cereal, has an alkaline ash and slight base balance.

3. Standard wheat bread has a distinct alkaline ash but the balance is acid. The alkalinity of the ash is due to the loss of chlorine from the sodium chloride used in baking.

4. Soybeans, jack beans, and potatoes have a distinct alkaline ash and a distinct base balance.

5. The loss of chlorine during ashing is correlated with the acid or base balance. The cereals with an acid balance lost all their chlorine during ashing; the other materials, with a base balance, retained practically all their chlorine.

6. The transformation of phosphorus into metaphosphate and pyrophosphate during ashing is inversely correlated with the alkalinity of the ash, especially when the alkalinity is recalculated on the uniform basis of one per cent phosphoric acid. Under the conditions of these experiments buckwheat, with an alkalinity value of about 13, gave 80 per cent of the phosphorus in the ash as orthophosphates; beginning with the alkalinity value of about 22 the entire phosphorus content of the ash is in the form of orthophosphate. The orthophosphate content of the ash in the cereals, with the exception of standard white flour bread, fluctuated from 7 to 54 per cent of the total phosphorus; the highest percentage of 54 was obtained from barley which had the highest alkalinity value of all the cereals on the recalculated basis of one per cent phosphoric acid.

7. The loss of sulphur during ashing is not correlated either with the acid-base balance of the materials or with the alkalinity values of the ash.

8. It is suggested that the significance of the acid-base balance, if any, besides its possible contribution to the acid-base equilibrium of the animal or human system, may lie also in its effect on the end products of mineral metabolism.

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LOSSES OF VITAMIN C DURING BOILING AND STEAMING OF CARROTS¹

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A survey of the literature shows only one study reporting a comparison of the losses of vitamin C during the boiling and steaming of vegetables. Since it has been found by Fenton, Tressler, Camp, and King (1937) that during the cooking of fresh peas about 50 per cent of the vitamin C is lost to the cooking water by solution, it seems probable that in steaming, where the vegetables are not immersed in water, the loss would be smaller. Carrots lend themselves well to both methods of cooking and were, therefore, chosen for this study in spite of the fact that they are known to be low in vitamin C. Raw carrots of unnamed varieties have been reported to contain .012, .017,³ .033,³ and .041 mg. of ascorbic acid per gram by McHenry and Graham (1935); Langley, Richardson, and Andes (1933); Wasson (1931); and Fellers (1935), respectively. The change in the content of this vitamin (owing to the boiling of carrots, as reported by these workers, varied from a loss of 50 per cent to a gain of 100 per cent. In a publication by Halliday and Noble (1936) a loss of 56 per cent from the carrots and a gain to the cooking water of 22 per cent during a 30-minute cooking period is reported. They do not give the actual vitamin C content of their samples. None of the above workers made a distinction between the unoxidized and reduced ascorbic acid with the exception of McHenry and Graham, who found .012 mg. of "free" and .009 mg. of "combined" ascorbic acid per gram of raw carrots.

The purpose of this study was to compare the losses of vitamin C from a known variety of carrots of known history when cooked by the boiling and steaming methods.

EXPERIMENTAL PROCEDURE

The Chantenay variety of carrots selected for this study was grown on Arkport fine sandy loam soil at Geneva, New York. They

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² Buhl Foundation Research Fellow. Publication No. 352 from the Chemistry Department, University of Pittsburgh.

³ Recalculated on the assumption that .5 mg. of ascorbic acid per day is required for the protection of a guinea pig from scurvy, Bessey and King (1933).

were pulled at the mature stage (about six carrots to the pound) each morning shortly before they were used and were prepared for cooking by washing and cutting crosswise in one-eighth-inch slices. This method of cutting gives a large amount of cut surface but is a very commonly used one, and it was necessary to have cooked pieces of such size and shape that they could be quickly immersed in acid in a weighing bottle.

Cooking in Boiling Water: This method was essentially that of Halliday and Noble (1933). The carrots, 325 grams or four servings, were plunged into 750 c.c. of rapidly boiling tap water containing one teaspoon of salt. A three-quart enamel pan with an inside diameter of seven inches was used. The water was boiled a short time before the carrots were added. The cover was left off to allow for removal of samples during the cooking. The evaporation in successive cookings was maintained at practically the same rate by means of a manometer, as described by Fenton, Tressler, Camp, and King (1937). The heat was so controlled that the water came back to the boil within two minutes after the carrots were added. It was found necessary to cook them 15 minutes.

The method of sampling the carrots and cooking waters, at intervals during the cooking periods, was the same as that reported by Fenton, Tressler, and King (1936). Samples were also taken at the done stage from cookings in which there had been no interference from previous sampling.

Steaming: An enamel steamer with a diameter of eight and one-half inches and an upper inset pan two and three-fourths inches deep was used. The entire steamer was hot and the water boiling when the 325 grams of sliced carrots were added. Different cookings were steamed 20 (done) and 25 (overdone) minutes. Samples of the carrots for vitamin C and moisture determinations were taken at the end of the cooking period only, since the cover was left on the steamer during the entire time.

The methods of extraction and titration were essentially those of Bessey and King (1933) as modified by Mack and Tressler (1937). A mixture of five per cent sulphuric and two per cent metaphosphoric acid was used for extraction. The dichlorophenolindophenol dye used was standardized daily with pure ascorbic acid. Blanks were run with the dye.

Since the body is able to use the dehydro as well as the reduced form of ascorbic acid, determinations were made of both the reduced and the reduced plus the reversibly oxidized ascorbic acid. The procedure employed in regeneration was that of Tillmans, Hirsch, and Jackisch (1932) with the following modification as to time: hydrogen

sulphide was bubbled through the extract for 10 minutes, the container was stoppered and allowed to stand for 20 minutes. The excess hydrogen sulphide was then removed with a stream of carbon dioxide and the extract titrated.

For the biological assay, samples of the sliced raw carrots were blanched in water at 100°C. (212°F.) for 120 seconds. Since it has been found that the freezing of vegetables causes no loss of vitamin C while blanching does cause loss, as noted by Fenton, Tressler, and King (1936), a larger quantity was necessary for feeding than would have been the case if the raw carrots had been frozen without blanching. The blanched and the cooked samples, which were cooled approximately four minutes in shallow pans surrounded by ice and salt, were packed, sealed, frozen, and stored in a container with dry

TABLE 1
*Vitamin C Assay of Blanched and Cooked Carrots
After Storage With Dry Ice*

Test food	Weight of carrots fed	Vitamin equivalent per day	Number of animals	Average weight at beginning of test	Average change in weight during test	Average scurvy score (0-24)
	gm.	mg.		gm.	gm.	
Cooked carrots.....	10	0.50	10	341	+74	3.6
Blanched carrots.....	12	0.55	10	338	+95	2.3
Vitamin solution.....	0.50	10	337	+75	3.6
Positive controls.....	1.00	3	332	+85	0.0
Negative controls.....	0.00	4	300	—98	18.0

ice. The curative method described previously by Tressler, Mack, and King (1936) was used with the exception that the curative feeding period was extended to 21 days.

The results of the assay (Table 1) show very close agreement in growth response and scurvy scores of the animals receiving the cooked carrots in comparison with those receiving standard vitamin solution. There was fair agreement in response of the animals receiving the blanched carrots.

DISCUSSION

The results of the vitamin C determinations are presented (Tables 2 and 3). The raw carrots contained an average of .044 mg. of ascorbic acid and .069 mg. of reduced plus reversibly oxidized ascorbic acid per gram. The former varied from .032 to .052 and the latter from .044 to .069 mg. per gram. Some of the ascorbic acid was apparently oxidized to the dehydro form during the very thin slicing of the raw carrots which was necessary to facilitate grinding. The vitamin C content both before and after reduction was greater than

that reported by other workers, .012 to .033 mg. per gram, determined by either the biological or chemical methods of determination. The higher value may be due to variety, growing conditions, maturity, or freshness.

At the end of the cooking period the boiled carrots retained .036 mg. of ascorbic acid per gram. There was some increase in titration value after regeneration of the carrot extract. It seems probable that this increase was due, however, to the formation of non-vitamin dye-reducing substances. Hence the values for the cooked carrots given in the tables are those obtained before regeneration. At the

TABLE 2
Vitamin C Loss From Carrots and Gain to Cooking Water

Length of cooking period ¹	Ascorbic acid						
	Drained carrots		Cooking water ⁴	In total drained carrots	In total cooking water	Per cent of original in	
	Wet weight	Dry weight				Carrots	Cooking water
min.	mg. per gm.	mg. per gm.	mg. per c.c.	mg.	mg.		
0 (raw)	.069 ²	.59	.000	22.4	0.0	100	0
2	.056	.47	.005
4006
6	.050	.45	.009
8	.047	.45	.010
10	.047	.44	.012
12	.043	.41	.016
15	.040	.38	.021
Samples not taken until carrots were done.							
0 (raw)	.064	.46	.000	20.8	0.0	100	0
15	.036 ³	.32	.017	11.6	6.8	56	33

¹ In all cases approximately two minutes were required to bring the water back to boiling after the carrots were put into it, hence the carrots cooked 15 minutes had been boiled 13 minutes. ² Obtained previous to reduction, .050. ³ When computed in terms of raw vegetable, .038. ⁴ Change in vitamin content of water owing to evaporation and removal of samples as well as solution from the vegetable.

end of the boiling period the cooking water contained .017 mg. of vitamin C per gram. There was practically no increase in titration value after treatment of the cooking water with hydrogen sulphide. This is in agreement with the data on the cooking water of carrots reported by Mack and Tressler (1937) with the use of the same extractants.

The total destruction of vitamin C was only 11 per cent, 56 per cent being retained in the carrots and 33 per cent in the cooking water. In determining the unoxidized acid Halliday and Noble (1936) found a total destruction of 34 per cent, 44 per cent being retained in the carrots and 22 per cent in the cooking water. The

carrots they used were purchased in the open market, were of unknown variety, and while they were cooked by essentially the same method, they were cooked a longer period of time (30 minutes instead of the 15 minutes found necessary to tenderize the freshly pulled Chantenay sliced carrots). The increase upon cooking from .012 to .026 mg. per gram of free ascorbic acid found by McHenry and Graham (1935) may be because (1) there was probably a considerable amount of the reversibly oxidized ascorbic acid formed during the grinding of the raw vegetable and this was not determined; (2) the so-called ascorbic acid oxidizing enzyme was inactivated during the

TABLE 3
Vitamin C Loss From Carrots by Steaming

Length of steaming period	Ascorbic acid			
	Drained carrots		In total carrots	Per cent of original in drained carrots
	Wet weight	Dry weight		
min.	mg. per gm.	mg. per gm.	mg.	
0 (raw)	.055 ¹	.41	17.9	100
20 (done)	.050 ²	.37	15.3	86
0 (raw)	.051 ³	.41	16.6	100
25 (over done)	.047	.37	14.1	85

¹ Obtained previous to reduction, .043. ² When computed in terms of raw vegetable, .038. ³ Obtained previous to reduction, .044

first few minutes of cooking; and (3) non-vitamin dye-reducing substances were formed during cooking.

At the end of the 20-minute cooking period the steamed carrots lost only 14 per cent and the "overdone" only 15 per cent of their vitamin C. This small decrease might be expected because (1) most of the loss during the boiling of the carrots was found to be by transfer into the cooking water and (2) the carrots were sliced thin so that the heat which inactivates the so-called oxidizing enzyme could penetrate quickly.

SUMMARY

1. Fresh raw carrots of the Chantenay variety contain from .044 to .069 mg. of vitamin C per gram.
2. Boiled sliced carrots contain about .036 mg. of vitamin C per gram or 56 per cent of their original vitamin C.
3. During a 15-minute cooking period almost two-fifths of the vitamin C present in carrots is dissolved in the water, but only about 11 per cent is destroyed.
4. Steaming carrots permits an 86 per cent retention of vitamin C.

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LOSSES OF VITAMIN C DURING COMMERCIAL FREEZING, DEFROSTING, AND COOKING OF FROSTED PEAS¹

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The vitamin C content of frosted² peas is of interest inasmuch as 16,000,000 pounds or one-half the frozen vegetable pack of 1936, were peas, according to Tressler (1937). Investigators report the vitamin C content of fresh raw peas from .14 to .46 mg. per gram, but there is little information available as to the vitamin C content of frosted vegetables. It is known that freezing alone does not affect the vitamin C content of peas, as noted by Fenton, Tressler, and King (1936), but frosted peas as they appear on the market have been subjected to a number of processes in addition to actual freezing. Such processes include blanching, cooling, packaging, sealing, and shipping and it may well be expected that one or several of these steps will result in loss of some of the vitamin C content.

One study on the vitamin C content of frosted peas found in the literature was made by Fellers and Stepat (1936) who found a decrease from .35 to .13 mg. of vitamin C per gram during the processes involved in preparing and marketing frosted peas. This marked difference may be due to sampling as well as to losses in blanching, cooling, etc. The vitamin C content of the fresh peas was based on 45 samples and of the frosted peas on only 13 samples which apparently were not paired with the fresh. During the defrosting of peas (two to six hours) they found a further loss to .041 mg. per gram. This large loss may be because (1) the so-called ascorbic acid-oxidizing enzyme was not completely inactivated during the short blanching period which was used and (2) no tests were made for any dehydroascorbic acid which might have been formed during the standing of the peas.

A report on the cooking losses of vitamin C from frosted peas was also made by these workers. They used a small amount of peas (140 grams) with a very small amount of water (60 grams) and their method involved evaporation of "practically all of the added

¹ Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 209, June 21, 1937.

² "Frosted" is a term applied to quick-frozen foods. The peas used in this study were furnished by the Birdseye Laboratories.

water." It should be noted that whatever small amounts of cooking water were left were not analyzed for vitamin C. Analyses in this laboratory of small amounts of cooking water unabsorbed by peas have indicated that it contains a high concentration of the vitamin. Thus, while the vitamin content of the peas themselves decreased, some of the vitamin in the above study would probably have been found in any small portion of cooking water if it had been analyzed. Moreover, the large proportion of peas to cooking water used in the study by Fellers and Stepat would involve a relatively long time to bring the water back to boiling after the addition of peas. Increasing the period of time of holding peas at this range of temperature would probably increase actual destruction of the vitamin. Furthermore, the peas used by these workers had been blanched, prior to freezing, by subjecting them for 40 seconds to steam at 100°C.(212°F.). It is doubtful whether this treatment would completely inactivate the enzymes. Consequently, owing to enzymic action, the vitamin C in these vegetables was subject to oxidation during the time it took the water to come back to the boiling point after the peas were added. Thus there were probably two factors contributing to the loss observed by these workers: (1) transfer of vitamin C from vegetable to cooking water and (2) actual destruction of vitamin C by enzymic oxidation permitted by the particular methods of blanching and cooking.

Recently, Jenkins, Tressler, and Fitzgerald (1937), in a study of the changes in ascorbic acid content of peas during commercial preparation and freezing, found a loss of 30 per cent of the total amount present, of which ten per cent occurred during blanching. The most rapid loss of ascorbic acid was found to occur during the cooling and washing operations subsequent to blanching. These workers also report that thawing peas results in no appreciable loss of vitamin C.

In view of the small amount of work which has been done on frosted peas it seemed worth while to investigate the following factors affecting the loss of vitamin C in these products: (1) current method of putting frosted peas on the market, (2) customary household holding of frosted peas, (3) cooking of frosted peas. Such a study will indicate the relative value of cooked fresh peas and cooked frosted peas as sources of vitamin C.

EXPERIMENTAL PROCEDURE

The Thomas Laxton variety of peas was used. The peas were shelled, washed, blanched 60 seconds in boiling water or 120 seconds in steam at 100°C.(212°F.), cooled, and frozen two hours at -17.8°C. (0°F.) in regular 10- or 12-ounce, wrapped, waxed cartons in a Birds-

eye Multiplate Freezer. They were packed in dry ice and shipped from Albion to Geneva, New York, and held at $-40^{\circ}\text{C}.$ ($-40^{\circ}\text{F}.$). At this temperature no loss in vitamin C content occurs, according to Fenton, Tressler, and King (1936).

Vitamin C determinations were made on the (1) raw fresh peas; (2) raw frosted peas (a) taken directly from storage at $-40^{\circ}\text{F}.$, (b) held in a refrigerator at $4.4^{\circ}\text{C}.$ ($40^{\circ}\text{F}.$) for 16 hours, (c) held at room temperature for one and five hours; (3) both raw and cooked frosted peas that had been (a) taken directly from storage at $-40^{\circ}\text{F}.$, (b) held in a refrigerator at $40^{\circ}\text{F}.$ for 16 hours; that is, cooked fresh peas were compared with cooked frosted peas as sources of vitamin C. A comparison was also made of the vitamin C content of peas from the same lot cooked (1) with the flame so regulated that the water came to a boil (a) in two and (b) in four minutes, (2) with the cooking utensil (a) uncovered, (b) covered.

The method of cooking the frosted peas was that given on the package. A three-quart enamel pan with an inside diameter of seven inches was used. The 283.5 grams of peas were added to 292 c.c. of rapidly boiling tap water containing one-half teaspoon of salt. An arbitrary stage of "doneness" was determined in advance by testing with a fork and "biting." It took eight minutes to reach this stage in all cases.

The rate of evaporation in successive cookings was controlled by means of a manometer as described by Fenton, Tressler, Camp, and King (1937). The heat was so controlled that the water in which the peas taken from storage at $-40^{\circ}\text{F}.$ and the water in which the peas taken from the refrigerator were cooked came back to the boil in four and two and one-half minutes, respectively. Peas from the same lot were cooked with the cooking utensil covered and uncovered.

The method of sampling the peas and cooking water at intervals during the cooking periods was the same as that used by Fenton, Tressler, and King (1936) on fresh peas. There was no interference from sampling in further cookings which were made to the done stages.

The methods of extraction, of standardization of the dye, and titration were essentially those of Bessey and King (1933) as modified by Mack and Tressler (1937). Since the peas had been frozen, and as a result were very easily crushed even in the raw state, the analysis of duplicate samples for ascorbic acid required less than one hour. A mixture of eight per cent of trichloroacetic and two per cent of metaphosphoric acid was used for extraction. Aliquots of the vegetable extracts and the cooking water were titrated with the 2, 6

dichlorophenolindophenol dye. The dye was standardized daily with pure ascorbic acid. Blanks were run with the dye. Since the body is able to use the dehydro as well as the reduced form of ascorbic acid, aliquots of the vegetable extracts and the cooking waters were immediately regenerated with hydrogen sulphide, according to the method of Tillmans, Hirsch, and Jackisch (1932) as modified by Mack and Tressler (1937). In view of the close agreement obtained between the chemical titrations and the biological assays of the two varieties of frozen unblanched peas and frozen cooked peas, as shown by Mack, Tressler, and King (1936) and Fenton, Tressler, and King (1936), it was not deemed necessary to check the chemical findings with a biological assay in this study.

TABLE 1
*Vitamin C Content of Fresh Peas, Frosted Peas,
and Frosted Peas Upon Standing*

Treatment	Temperature	Ascorbic acid	
		Wet weight	Dry weight
		<i>mg. per gm.</i>	<i>mg. per gm.</i>
Raw, unfrozen	Fresh	.25	1.05
Uncooked, frosted	Weighed in $-17.8^{\circ}\text{C.}(0^{\circ}\text{F.})$ room.	.18	0.65
Uncooked, frosted	Taken directly from 0°F. room but weighed in room at $26.7^{\circ}\text{C.}(80^{\circ}\text{F.})$.	.18	0.65
Uncooked, frosted	Held in an electric refrigerator at $4.4^{\circ}\text{C.}(40^{\circ}\text{F.})$ for 16 hours.	.18	0.65
Uncooked, frosted	Held at room temperature 80°F. for 1 hour.	.18	0.65
Uncooked, frosted	Held at room temperature 80°F. for 5 hours.	.18	0.65

DISCUSSION

The effects of commercial freezing and of defrosting frosted peas on the vitamin C content are given (Table 1). The results of cooking the frosted peas (1) taken directly from -40°F. and (2) held in the refrigerator are shown (Tables 2 and 3).

No increase in the titration value was obtained after treatment of the vegetable extracts with hydrogen sulphide, probably because the blanching periods were sufficient to inactivate the so-called ascorbic acid oxidizing enzyme.

The vitamin C content of the raw peas, about .25 mg. per gram, was the same as that obtained by biological assay of an unnamed variety of peas by Eddy, Kohman, and Carlsson (1926) and is also about the same as that reported for the Thomas Laxton variety (.23 mg. per gram) by Fenton, Tressler, and King (1936). It is higher

than the .16 mg. per gram reported by McHenry and Graham (1935) for an unnamed variety of peas.

The frozen peas contained .18 mg. of vitamin C per gram wet weight, or a loss of about 38 per cent. This loss must be due to blanching, chilling, and packaging since no loss occurs during the actual freezing and holding of peas at such low temperatures, according to Fenton, Tressler, and King. Blanching is probably more largely responsible for the loss than are the other processes, since

TABLE 2
Vitamin C Losses From Frosted Peas Taken Directly From Storage at $-17.8^{\circ}\text{C}.$ ($0^{\circ}\text{F}.$), and Gain to Cooking Water

Cooking period ¹	Ascorbic acid						
	Drained peas ²		In cooking water ⁴	Total in		Original in	
	Wet weight	Dry weight		Drained peas	Cooking water	Drained peas	Cooking water
min.	mg. per gm.	mg. per gm.	mg. per c.c.	mg.	mg.	per cent.	per cent.
0 (uncooked)	.18	.65	.00
3	.15	.60	.02
4	.14	.59	.02
5	.13	.56	.04
6	.13	.56	.05
7	.12	.54	.06
8 (done)	.11	.52	.08
No sampling until peas were done.							
0 (raw)	.17	.62	.00	48.0	0.0	100	0
8 (done)	.12 ³	.50	.07	28.5	17.3	59	36
10 (overdone)	.11	.52	.09
12 (overdone)	.12	.54	.11
14 (overdone)	.14	.60	.16

¹ Approximately four minutes were required to bring the water back to boiling after the frosted peas were added. ² The values for the cooked peas were computed on the wet weight of the cooked samples. ³ When calculated in terms of the uncooked peas this value is .10. ⁴ Change in vitamin content of water owing to evaporation and removal of samples as well as solution from the vegetable.

in the cooking of fresh peas the greatest loss of vitamin C occurs during the first two minutes, according to Fenton, Tressler, and King.

When frosted peas were taken directly from storage at $-40^{\circ}\text{C}.$ ($-40^{\circ}\text{F}.$) and then held in cartons (1) at room temperature ($80^{\circ}\text{F}.$) with the seal broken for one and five hours, (2) in a household electric refrigerator at $40^{\circ}\text{F}.$ for 16 hours with the seal unbroken, there was no appreciable loss of vitamin C. Holding frosted peas, except at below freezing temperatures, however, is not advisable because of spoilage which may occur. The rapid loss of vitamin C during the defrosting of frosted peas, observed by Fellers and Stepat

(1936), may be because the so-called ascorbic acid-oxidizing enzyme was not inactivated during the blanching of the raw peas.

During cooking there was very little destruction of vitamin C but only a loss to the cooking water. This was true whether the peas were taken directly from storage at -40°F. or from the refrigerator. This slight destruction, even after holding, may be because the so-called ascorbic acid-oxidizing enzyme was inactivated during the blanching. At the end of the cooking period the peas taken directly from storage at -40°F. retained 59 per cent of their vitamin C while 36 per cent had been transferred to the cooking water; and

TABLE 3
Vitamin C Losses From Frosted Peas Stored Overnight (16 Hours) in a Refrigerator $4.4^{\circ}\text{C.}(40^{\circ}\text{F.})$, and Gain to Cooking Water

Cooking period ¹	Ascorbic acid						
	Drained peas ²		In cooking water ⁴	Total in		Original in	
	Wet weight	Dry weight		Drained peas	Cooking water	Drained peas	Cooking water
min.	mg. per gm.	mg. per gm.	mg. per c.c.	mg.	mg.	pct.	pct.
0 (uncooked)	.17	.76	.00
3	.13	.48	.04
4	.12	.47	.05
5	.12	.47	.05
6	.11	.46	.06
7	.10	.46	.07
8 (done)	.10	.46	.08
No sampling until peas were done.							
0 (raw)	.17	.76	.00	48.0	0.0	100	0
8 (done)	.12 ³	.54	.08	27.0	18.7	56	39

¹ Approximately two and one-half minutes were required to bring the water back to boiling after frosted peas were added. ² The values for the cooked peas were computed on the wet weight of the cooked samples. ³ When calculated in terms of the uncooked frosted peas this value is .10. ⁴ Change in vitamin content of water owing to evaporation and removal of samples as well as solution from the vegetable.

the peas held in the refrigerator retained 56 per cent while 39 per cent had been transferred to the cooking water. At the end of a 16-minute cooking period fresh peas of the same variety retained 42 per cent of their vitamin C while 44 per cent had been transferred to the cooking water.

The vitamin C content of the cooked frozen peas, .12 mg. per gram, compares very favorably with that of the cooked fresh peas of the same variety, .11 mg. per gram, as reported by Fenton, Tressler, and King (1936). The cooking water of the frosted peas contained less vitamin C, .07 to .08 mg. per c.c., than did that of the fresh peas, .13 mg. per c.c. Both these differences may be partially explained by

the fact that the frosted peas required only eight minutes and the fresh peas 16 minutes of cooking. It seems probable that, in general, cooked frosted peas compare very favorably with cooked fresh peas, particularly when the latter are purchased on the open market, for Mack, Tressler, and King (1936) found that fresh peas lose about one-half of their vitamin C content when held at room temperature for three days.

Having the kettle covered or uncovered seemed to make no difference in the loss of vitamin C from the frosted peas as in both cases of eight minutes' cooking the decrease was from .15 to .11 mg. per gram. Having the water come back to boiling in two minutes or four minutes made no difference in results; in both cases the decrease was from .16 to .12 mg. per gram. The above findings may be partially explained on the basis that the so-called ascorbic acid-oxidizing enzyme is inactivated during the blanching and that most of the loss of the vitamin from the peas is to the cooking water. This transference would probably not be affected by the kettle being covered or uncovered or by the slightly longer time required for the water to come to the boil, since in each case the peas were cooked for the same length of time.

SUMMARY

1. The vitamin C content of fresh peas of the Thomas Laxton variety was reduced from .25 to .18 mg. per gram wet weight or a loss of about 38 per cent during the processes, other than actual freezing, involved in putting the peas on the market in the frosted form (blanching 60 seconds in boiling water or 120 seconds in steam at 100°C.(212°F.), cooling, packaging, sealing, and shipping.

2. The vitamin C content of frosted peas remained practically the same when they were taken directly from storage at -40°C. (-40°F.) and held (1) in an electric refrigerator at 4.4°C.(40°F.) for 16 hours or (2) at room temperature (80°F.) for one and five hours.

3. The cooked frosted peas, which were taken directly from storage at -40° C., retained 59 per cent of their vitamin C and 36 per cent was dissolved in the cooking water. The cooked frosted peas which had been held in the refrigerator retained 56 per cent of their vitamin C, and 39 per cent was dissolved in the cooking water.

4. The vitamin C content of the cooked frosted peas studied was about the same (.12 mg. per gram) as the cooked fresh peas of the same variety (.11 mg. per gram) determined in a previous study.

5. The vitamin C content of the cooking water at the end of the cooking period was .07 to .08 mg. per cubic centimeter.

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ISOLATION OF HALOPHILIC BACTERIA FROM SOIL, WATER, AND DUNG

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Although it has been claimed by Clayton and Gibbs (1927); Robertson (1931); and Zobell, Anderson, and Smith (1937) that halophilic bacteria are distinct species indigenous to environments containing high concentrations of sodium chloride, work in this laboratory by Stuart, Frey, and James (1933) and Stuart and Swenson (1934) has indicated that such bacteria are adapted forms of ordinary bacteria known to be indigenous to other environments, such as stagnant water, sulphur springs, soil, and dung.

It is well known that bacteria growing on media containing 25 per cent or more of sodium chloride have exceedingly long lag periods of from 20 to 30 days when they are cultivated directly from a high salt environment, such as reddened salted hides or fish. Lochhead (1934); the Salton Sea, Pierce (1914); the Great Salt Lake, Zobell, Anderson, and Smith (1937); the Dead Sea, Willansky (1936); or the solar evaporation pools used in the commercial production of sea salt. Thus it seems only reasonable to suppose that the cultivation of similar organisms from environments with a very low concentration of sodium chloride might necessitate even longer incubation periods before demonstrating visible growth. This point seems to have been overlooked by recent workers who have attempted to cultivate bacteria on high salt media from native material of a low salt content.

Our laboratory experiments have shown that this is often so, and by employing incubation periods of from 60 to 90 days chromogenic bacteria, similar to those reported as being responsible for the reddening of salted hides, have been isolated on agar containing 25 per cent of sodium chloride from stagnant water, sulphur springs, freshly flayed calfskin, rat dung, and four samples of native soils representing different geographical locations within the United States.

Large test tubes containing 25 ml. of nutrient brine (25 per cent c.p. NaCl, 1 per cent peptone, .5 per cent CaCl₂, and .5 per cent MgSO₄·7H₂O in distilled water) were sterilized in an autoclave for 20 minutes at 15 pounds pressure and then inoculated with one-milliliter aliquots of the water samples to be tested and two-gram

TABLE 1

*Isolation of Red Halophilic Bacteria From Sources of
Low Sodium Chloride Content*

Source	Isolations on milk-salt agar after incubation in nutrient brine				
	0 days	30 days	60 days	90 days	
1. Water from stagnant pool in marsh, D. C.....	No growth	No growth	No growth	Growth	Growth
2. Water from Black Sulphur Springs, Sulphur Springs, Ark.....	No growth	No growth	No growth	Growth
3. Water from Sulphur Springs, Lake Hill Park, St. Louis, Mo.....	No growth	No growth	Growth
4. Water from Petty's Sulphur Spring Valley Park, St. Louis, Mo.....	No growth	No growth	No growth	No growth	No growth
5. Water from Sulphur Springs, Sulphur Springs Rd., St. Louis, Mo.....	No growth	No growth	No growth	No growth	Growth
6. Calfskin, fresh, from cooler, abattoir, D. C.....	No growth	No growth	No growth	Growth	Growth
7. Calfskin, fresh, from cooler, abattoir, D. C.....	No growth	No growth	No growth	Growth	Growth
8. Rat dung from laboratory, U.S.D.A.....	No growth	No growth	No growth	Growth	Growth
9. Rat dung from laboratory, U.S.D.A.....	No growth	No growth	No growth	Growth	Growth
10. Rabbit dung from laboratory, U.S.D.A.....	No growth	No growth	No growth	No growth	No growth
11. Rabbit dung from laboratory, U.S.D.A.....	No growth	No growth	No growth	No growth	No growth
12. Native soil, Portsmouth fine sandy loam, N. C.....	No growth	No growth	No growth	No growth	No growth
13. Native soil, Herman soil, N. H.....	No growth	No growth	No growth	No growth	No growth
14. Native soil, Pierre clay loam, S. D.....	No growth	No growth	No growth	No growth	Growth
15. Native soil, Cermian clay loam, S. D.....	No growth	No growth	No growth	Growth	Growth
16. Native soil, Niobra clay loam, Logan County, Kansas.....	No growth	No growth	No growth	No growth	No growth
17. Native soil, Bressua loam, N. H.....	No growth	No growth	No growth	No growth	No growth
18. Native soil, Benton shale, Las Animas, Colo.....	No growth	No growth	No growth	No growth	Growth
19. Native soil, Pecosan muck, N. C.....	No growth	No growth	No growth	No growth	No growth
20. Native soil, Colby (Hays) clay loam, Kansas.....	No growth	No growth	No growth	No growth	No growth
21. Native soil, sandy loam, Gloucester, Mass.....	No growth	No growth	No growth	No growth	No growth
22. Native soil, Norfork fine sandy loam, N. C.....	No growth	No growth	No growth	No growth	No growth
23. Native soil, Dundbar fine sandy loam, N. C.....	No growth	No growth	No growth	No growth	No growth
24. Native soil, Bladen fine sandy loam, N. C.....	No growth	No growth	No growth	No growth	No growth
25. Native soil, silt loam, Colfax Co., N. M.....	No growth	No growth	No growth	No growth	Growth
Six sterile control brines.....	No growth	No growth	No growth	No growth	No growth

portions of the freshly flayed calfskin, dung, and soil samples, respectively. Aliquots of one ml. were removed from all sterile uninoculated control tubes and the inoculated brines before and after incubation for 30, 60, and 90 days and plated in a modified Lochhead's (1934) milk-salt agar (25 per cent NaCl).¹ The plates were then incubated at 30°C. (86°F.) for 30 days and all growths recorded. During incubation of the tubes cotton plugs were pushed in beyond the lip and corks inserted to prevent excessive evaporation of the brine.

After the 30-day incubation period all growths found on the agar plates were transferred to a series of agar slants of the same organic composition but containing 25, 22.5, 20, 17.5, 15, and 12.5 per cent, respectively, of sodium chloride to determine the optimum concentration of salt for the growth of the organism. Only those organisms that grew better on the agar containing 25 per cent of sodium chloride than on those containing lesser percentages and only chromogenic bacteria are reported (Table 1).

Morphological studies of the individual cultures reported (Table 1) showed seemingly pure strains of large, encapsulated gram-negative cocci with primary diplococcal arrangements for the soil samples No. 14, 15, 18, and 25; small gram-negative bacterial rods for the samples of water No. 1, 2, and 5; irregular gram-negative cocci-rod complexes from the calfskin and rat dung, samples No. 6, 7, 8, and 9; and an apparently pure strain of gram-negative thread forms from spring water sample No. 3. The thread-form culture was particularly interesting owing to its tendency to grow in characteristic isolated colonies.

These results show definitely that halophilic bacteria are widely distributed in nature and can be cultivated from sources other than salt or concentrated brines if allowances are made for exceedingly long lag periods. They also explain the frequent appearance of flesh reddening on old salted hides and skins known to have been salted originally with uncontaminated salt. It is of further interest in this connection that the 90-day lag period found in these studies corresponds closely to the time that usually elapses before active bacterial growth will appear on pieces of freshly flayed skin salted with an excess of uncontaminated C.P. salt and incubated in culture dishes in the laboratory.

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¹ Two per cent bacto-gelatine added.

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DETECTING THERMOPHILIC CONTAMINATION IN SKIM-MILK POWDER

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Increasing numbers of uses for milk powders particularly in the canning industry are placing more stringent standards upon the manufacturer of this product. The canner has long been familiar with the difficulties resulting from contamination of canned foods particularly with heat-resistant types of microorganisms. Thermophiles in concentrated or dried milks are not uncommon. This is easily understood in view of the prolonged heat treatment necessary in the manufacture of these products.

According to Cameron (1936) since 1926 the canning industry has been aware that both beet and cane sugar may carry spores of all three groups of thermophilic bacteria which are important agents in the spoilage of non-acid canned foods. This author lists the three groups as represented by flat-sour bacteria (*Bacillus stearothermophilus*); thermophilic anaerobes, not producing hydrogen sulphide (*Clostridium thermosaccharolyticum*); and sulphide-spoilage bacteria (*Clostridium nigrificans*). Of these the flat-sour group is the most common and is the type concerned in the present study.

Cameron gives the following description of flat-sour colonies on dextrose-tryptone agar: "... colonies are characteristic. The colony is round, measures from 2 to 5 mm. in diameter, presents a typical opaque central 'spot', and by reason of acid production in the presence of the indicator is usually surrounded by a yellow halo in a field of purple. The halo may be insignificant or missing where certain low acid producing types are concerned or where the plate is so thickly seeded that the entire plate takes on a yellow tinge. The typical subsurface colonies are rather compact and may approach the 'pinpoint' condition."

Canned food spoilage from this type of organism is particularly troublesome because of the fact that it cannot be detected until the can is opened, as there are no outward signs of spoilage such as occur in gaseous spoilage where cans may swell even to the point of leakage.

Whether or not the thermophilic spores encountered in milk powders are identical with those encountered in sugars and starches (flat-sour organisms) has not been established at this writing. Neverthe-

¹ Now with National Butter Company, Dubuque, Iowa.

less, when milk powders are found which produce positive results when submitted to examination for flat-sours there is cause for concern on the part of the user of such products, especially if they are to be used in the preparation of canned soups and the like.

The present study is an outgrowth of work previously conducted by this laboratory and by the National Canners Association research laboratory on methods of ascertaining the presence of thermophilic and thermoduric microorganisms in milk powders which might be deleterious to the quality of canned foods if introduced in appreciable numbers.

The method employed in examining the series of samples herein reported was evolved from that specified by the National Canners Association for the examination of sugar ² for the so-called flat-sour organisms which in brief calls for:

- (a) Twenty grams of sugar made up to 100 c.c. volume with sterile distilled H₂O and boiled for five minutes.
- (b) Pipetting two c.c. of solution to each of five plates and pouring with Bacto-dextrose-tryptone agar.
- (c) Incubating the plates 36 to 48 hours at 55°C. (131°F.), counting colonies on five plates and converting the total to count per 10 grams of sugar.

Certain difficulties were encountered in adapting the above procedure to milk powders, and changes were made to accommodate them. It was found that 20 grams of milk powder in 100 c.c. of water gave a very unsatisfactory sample for plating, being too viscous, and when plated gave too great an amount of turbidity to the plates. Hence the amount of sample used was reduced to 10 grams per 100 c.c. The milk-powder solutions foamed badly upon attempts to boil them. Hence, upon advice of the National Canners Association Research Laboratory, exposure of the sample to autoclaving at five pounds steam pressure for 10 minutes was substituted for five minutes' boiling. Further, two c.c. of the milk-powder solution per plate caused too much turbidity to be readily countable and also interfered with the usefulness of the indicator in the media in detecting acid production. For these reasons only one c.c. of the sample was placed on each plate and 10 plates were used, thus accommodating one gram of the original powder sample.

ALKALINE-DILUTION BLANKS

Particular difficulty from undissolved powder and heat-coagulable material was encountered upon heating prior to plating in the present study when water dilutions were used.

² A detailed report on methods of examination of sugar was published by Cameron (1936, 1936a).

Prickett and Miller (1936) studied the use of alkaline-dilution blanks in bacteriological analysis of milk powders as a means of obtaining satisfactory solutions of the more highly acid types of powders. These investigators used N/10 and N/5 solutions of sodium and lithium hydroxides as dilution media and obtained counts comparable to those obtained in water dilutions of the same samples. Also more readily countable plates were obtained when alkaline blanks were employed for flakes and specks of powder which commonly appeared in water dilutions but were not present on plates made from the alkali dilutions.

In the present study a series of trials demonstrated that as little as N/60 sodium or lithium hydroxide completely eliminated this difficulty in the heat-treated dilutions and gave readily countable plates which were free from flakes and specks of undissolved material. In addition, it was observed that definite benefit in discerning acid-producing colonies resulted from the use of alkaline blanks, for many of the powders examined were sufficiently acid to affect the indicator in the medium when water dilutions only were plated. With alkali dilutions, however, a darker plate resulted which showed much more clear-cut reactions on acid-producing colonies.

SAMPLES STUDIED

The milk powders examined in this study consisted of 13 atmospheric-roller process and two spray-process skim-milk powders obtained from six different plants over a six-months period from February to July, 1936.

METHODS OF PROCEDURE

Since it was desirable to report thermophilic spore counts on a gram-of-powder basis, the proportion recommended by Prickett and Miller (1936), namely, 10 grams milk powder to 93 c.c. sterile water or alkali, was used to obtain a 1:10-dilution.

A preliminary trial conducted to determine the strength of alkali needed to give complete solution of roller-process powder upon autoclaving at five pounds steam pressure for 10 minutes demonstrated that N/50 and N/60 sodium or lithium hydroxides were the strongest which could be used without giving appreciable discoloration upon heating and also would yield a satisfactory solution of the powder.

In order to determine the effect of N/60 alkali upon the acidity and pH of diluted samples a 1:10 dilution of each sample was made with (1) distilled water, (2) N/60 NaOH, and (3) N/60 LiOH. Ten grams of the solutions were titrated with N/10 NaOH to phenolphthalein and the pH was determined electrometrically by means of

a quinhydrone electrode. The samples were then submitted to heat treatment at five pounds steam pressure for 10 minutes, cooled, and again titrated and the pH determined as before. Aseptic precautions were used throughout these manipulations.

The total thermophile counts were obtained by plating the water and alkali dilutions of each sample in serial dilutions of 1:10, 1:100, and 1:1000 before autoclaving and pouring with dextrose-tryptone agar.³ The development and use of this medium is described by Williams (1936). The thermophilic-spore and flat-sour-spore counts were obtained by autoclaving the 1:10 dilutions at five pounds steam pressure for 10 minutes, placing one c.c. of material on each of 10 plates per sample, and pouring with dextrose-tryptone agar. All plates were incubated at 55°C.(131°F.) for 36 to 48 hours and the count per gram of powder determined by counting the suitable dilution in the case of the total thermophile count and by counting the total colonies on 10 plates in the case of the thermophilic-spore counts.

The method suggested for the examination of skim-milk powder for thermophiles and thermophilic spores is as follows:

- (1) Weigh 10 grams of the powder into 93 c.c. of sterile sodium hydroxide in a Pyrex bottle or flask.
- (2) Shake vigorously to dissolve the powder.
- (3) Plate serial dilutions and pour plates with dextrose-tryptone agar.
- (4) Autoclave original dilution (1) at five pounds steam pressure for 10 minutes.
- (5) Using a 10-c.c. graduated pipette, plate one c.c. of autoclaved sample on each of 10 plates and pour with dextrose-tryptone agar.
- (6) Incubate plates at 55°C.(131°F.) for 36 to 48 hours.
- (7) Count total colonies on 10 plates and record total as thermophilic spores per gram, noting acid-producing colonies particularly.
- (8) Determine total thermophile count from (3) by counting suitable dilution, and record as total thermophiles per gram.

From the data secured on the 15 samples of skim-milk powder examined it is apparent that the pH of the samples diluted either in water or alkali dropped slightly after heat treatment (Table 1). The titration values increased in most cases in both water and alkali dilutions, but far greater increases occurred in the case of the alkali dilutions.

³ This medium is recommended by the National Canners Association research laboratory and is obtainable in dehydrated form from Digestive Ferments Company, Detroit, Michigan. The formula is as follows:

Bacto-tryptone.....	10.0 gm.
Bacto-dextrose.....	5.0 gm.
Bacto-agar.....	15.0 gm.
Bacto-brom-cresol-purple.....	0.04 gm.

Thirty grams of this formula dissolved in one liter of distilled water and autoclaved at 20 pounds pressure for 15 minutes has a final pH of 6.7±.

TABLE 1

Effect of Autoclaving 1:10 Dilutions of Skim-Milk Powder at Five Pounds Steam Pressure for 10 Minutes Upon the pH, Acidity, and Thermophile Content

Before autoclaving					After autoclaving		
No.	Dilution	pH	Acidity ¹	Thermo- philes per gm.	pH	Acidity ¹	Thermo- philic spores per gm.
1	H ₂ O	6.42	1.90	70	6.28	2.00	1
	N/60 NaOH	7.41	0.80	100	7.28	1.10	4
	N/60 LiOH	7.38	0.80	120	7.33	1.05	3
2	H ₂ O	6.39	1.80	110	6.28	1.95	29
	N/60 NaOH	7.52	0.80	110	7.28	1.00	73
	N/60 LiOH	7.47	0.70	120	7.23	1.00	77
3	H ₂ O	6.32	2.15	120	6.23	1.90	8
	N/60 NaOH	7.42	0.85	110	7.18	1.10	26
	N/60 LiOH	7.42	0.90	120	7.18	1.15	24
4	H ₂ O	6.41	1.95	110	6.26	1.90	9
	N/60 NaOH	7.51	0.80	140	7.28	1.15	6
	N/60 LiOH	7.42	0.85	150	7.23	1.20	6
5	H ₂ O	6.39	2.00	120	6.28	2.00	17
	N/60 NaOH	7.49	0.80	120	7.25	1.15	32
	N/60 LiOH	7.40	0.80	110	7.22	1.15	34
6	H ₂ O	6.32	2.00	7,400	6.21	2.00	2,480
	N/60 NaOH	7.34	0.85	11,200	7.17	1.00	2,460
	N/60 LiOH	7.31	0.75	8,400	7.14	1.20	2,640
7	H ₂ O	6.42	1.75	1,100	6.32	1.95	80
	N/60 NaOH	7.47	0.75	2,200	7.31	1.05	103
	N/60 LiOH	7.46	0.90	1,200	7.31	1.00	83
8	H ₂ O	6.29	2.05	60	6.25	2.05	1
	N/60 NaOH	7.52	0.80	70	7.17	1.40	1
	N/60 LiOH	7.43	0.70	70	7.08	1.35	3
9	H ₂ O	6.42	2.00	300	6.30	2.15	145
	N/60 NaOH	7.43	0.80	240	7.08	1.30	195
	N/60 LiOH	7.45	0.80	290	7.12	1.20	222
10 ²	H ₂ O	6.43	1.85	400	6.32	2.00	10
	N/60 NaOH	7.43	0.65	200	7.19	1.05	17
	N/60 LiOH	7.53	0.65	1,000	7.20	0.90	16
11	H ₂ O	6.49	2.05	20	6.27	2.15	20
	N/60 NaOH	7.51	0.90	30	7.10	1.20	21
	N/60 LiOH	7.48	0.95	30	7.15	1.20	19
12	H ₂ O	6.46	2.05	400	6.23	2.00	47
	N/60 NaOH	7.62	0.70	1,000	7.23	1.05	57
	N/60 LiOH	7.51	0.75	1,200	7.18	1.00	56
13 ²	H ₂ O	6.32	1.80	31,000	6.28	2.00	34,500 ²
	N/60 NaOH	7.69	0.50	41,000	7.25	1.00	49,100 ²
	N/60 LiOH	7.50	0.60	23,000	7.18	0.95	48,300 ²
14	H ₂ O	6.52	2.15	80	6.27	2.20	2
	N/60 NaOH	7.44	0.70	30	7.10	1.20	1
	N/60 LiOH	7.48	0.80	20	7.14	1.30	3
15	H ₂ O	6.33	2.20	1,000	6.18	2.00	141
	N/60 NaOH	7.30	1.00	900	7.01	1.25	188
	N/60 LiOH	7.40	0.90	900	7.08	1.20	197

¹ Acidity is recorded as c.c. of N/10 NaOH per gram of skim-milk powder. ² Samples 10 and 13 are spray-process powders. All others are atmospheric roller-process powders. ³ Ten c.c. of a 1:1000 dilution were used in obtaining the thermophilic spore count on this sample.

The highest pH obtained using N/60 alkali as a diluting medium was 7.67 (Sample 13), the final reaction after heating being pH 7.25. By comparing the counts obtained in water with those obtained in alkali blanks before heating, it will be noted that in most cases slightly higher counts resulted from the alkali dilutions. Even greater differences in favor of the alkali blanks is apparent after heating the samples. This, of course, may be explained largely by the more complete solution and subsequent freeing of organisms entrapped in powder flakes when alkali dilutions are used. These facts appear to eliminate the fear of detrimental effects of the alkali upon the viability of the organisms when employed in small concentrations.

With respect to preference in the case of the alkalies used, there seems to be little difference in favor of either and it appears that these differences may be attributed to experimental error in plating manipulations. The same may be said with regard to small differences in acidity-titration values since it is difficult to read the phenolphthalein end-point in milky solutions with a high degree of accuracy.

More spray-process powders might have been included in the present work but for the fact that ordinarily little difficulty in dissolving them in water is encountered. The use of alkali dilutions for spray powders is to be recommended, however, when a medium containing an indicator such as brom-cresol-purple is employed; as the relatively low pH of the average powder solution (1:10 dilution) does affect the usefulness of the indicator adversely when water dilution methods are employed.

CONCLUSIONS

On the basis of the foregoing data and observations the following conclusions seem warranted:

1. No detrimental effects on the thermophilic counts of 15 samples of skim-milk powder were observed when N/60 sodium or lithium hydroxides were employed as dilution media in place of distilled water.

2. On the whole slightly greater thermophilic spore counts were observed on plates made from N/60 alkali dilutions than were obtained from water dilutions of the same powders treated in the same fashion.

3. The common difficulty in counting plates from atmospheric roller-process powders owing to undissolved flakes and specks of undissolved material was eliminated when N/60 alkali was employed in place of ordinary distilled water as a diluting medium when dilutions were heat-treated prior to plating.

4. Definite advantages in using N/60 sodium or lithium hydroxide dilution blanks can be secured when plating skim-milk powders on dextrose-tryptone agar containing brom-cresol-purple as the pH of the average milk powder solution (1:10) is sufficiently low to impair the usefulness of the indicator in detecting acid-producing colonies when ordinary water-dilution methods are employed.

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COLOR IN CALIFORNIA WINES

I. METHODS FOR MEASUREMENT OF COLOR

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Although the eye is the ultimate judge of the beauty and attractiveness of color in a wine, it is not a particularly sensitive means for measuring color. The chief difficulties lie in the limitations of the eye alone as a recorder and of the observer to describe the sensation which a color produces. Moreover, the optical stimulus produced by the same color will vary with different individuals. The eye can evaluate two colors, however, in terms of certain primary stimuli and by analogy may compare the stimuli from the two sources. Thus by the proper mixing of three primary standards it is possible to duplicate the effect of a certain color. The International Commission on Illumination has standardized the amount of each of the primary standards necessary to produce the color represented by each wavelength of the visible spectrum. The values so determined are known as the "tristimulus values" of the spectrum colors. By means of the spectrophotometer and the proper transformations it is possible to measure the tristimulus values of a colored liquid, such as a wine. Complete information concerning this transformation and the subsequent calculations which can be made from the tristimulus data are given by Hardy (1936).

Specification of a color in terms of the tristimulus values does not, however, completely define it. Thus, although the quality of two colors may be the same, the amount of light which they transmit may be very different. If the exact nature of the pigments which produce the color in wines were known, then it would be possible to specify the color of a wine directly in terms of the amount of pigment based on the usual measurement of transmission factors and the use of Beer's law, other factors being equal. Until such information becomes available, it will be necessary to rely on the measurement of color as supplied by the spectrophotometer if a complete analysis is desired, or on certain other methods, for approximate specification.

The relative brightness of a colored surface or liquid varies respectively with the amount of light reflected or transmitted. A piece of white paper or a very thin and white liquid would have a brightness of nearly 100 per cent, while a black body or liquid would have a brightness of 0 per cent.

There is some confusion with respect to the use of the terms brightness and brilliance in wines. Since the brilliance of a wine has meant in enology the freedom from suspended material of a sample, we prefer to restrict use of the term brilliance to this meaning so far as wines are concerned. Brightness may then be used in its more inclusive sense to indicate variation in transmission of light through a liquid. Thus white wines have a fairly high brightness while darker red wines are of low brightness. This is quite irrespective of the use of the term brilliance of white and red wine, both of which may be free of suspended material and commercially would be called brilliant. It will be recognized, however, that where the amount of suspended material is large there will be a reduction in the transmission of light and a consequent diminution of the brightness. In spite of the fact that the two terms are thus interrelated it is important that they should not be confused; moreover, when relative brightness is being measured the wines compared must be brilliant.

The other terms necessary for complete specification of color are purity, which refers to degree of saturation of the color, and dominant wave-length, which indicates the particular hue of the sample. Although these definitions have psychological connotations, it is necessary to finally express them all in terms of physical standards if comparative results are to be obtained.

An intensive study of the color specification for wines has recently been published by Boutaric, Ferré, and Roy (1937). For a color characteristic of a wine they recommend using the optical density at 520 m μ and the quotient of the optical densities measured at 480 m μ and 640 m μ . For red wines having similar transmission curves this does suffice to distinguish between them. The values obtained, however, do not represent any function of color and are therefore difficult to interpret in terms of the complete color specification of a wine.

EXPERIMENTAL PROCEDURE

In the present study we have made use of a Bausch and Lomb Universal type spectrophotometer (No. 1200). The transmission curve of the sample was determined at intervals of 10 Å throughout as large a range of the visible spectrum as possible. Using the methods given in the Handbook of Colorimetry the tristimulus values of the sample were then calculated. This transformation was performed using the illumination data for Illuminant A, since this approximated the spectral distribution of energy of the tungsten light employed. Since the tristimulus value for the yellow primary has been made to fit the visibility curve of the eye, it is possible to determine the brightness of the sample directly by taking the tristimulus value for yellow

obtained by actual measurement and dividing it by the theoretical tristimulus value for the same range if the brightness were 100 per cent. The other color values, purity and dominant wave-length, are obtained from graphical plots by the use of the trichromatic coefficients. In order to indicate results obtained with this method some actual transmission curves are given (Fig. 1) together with trichro-

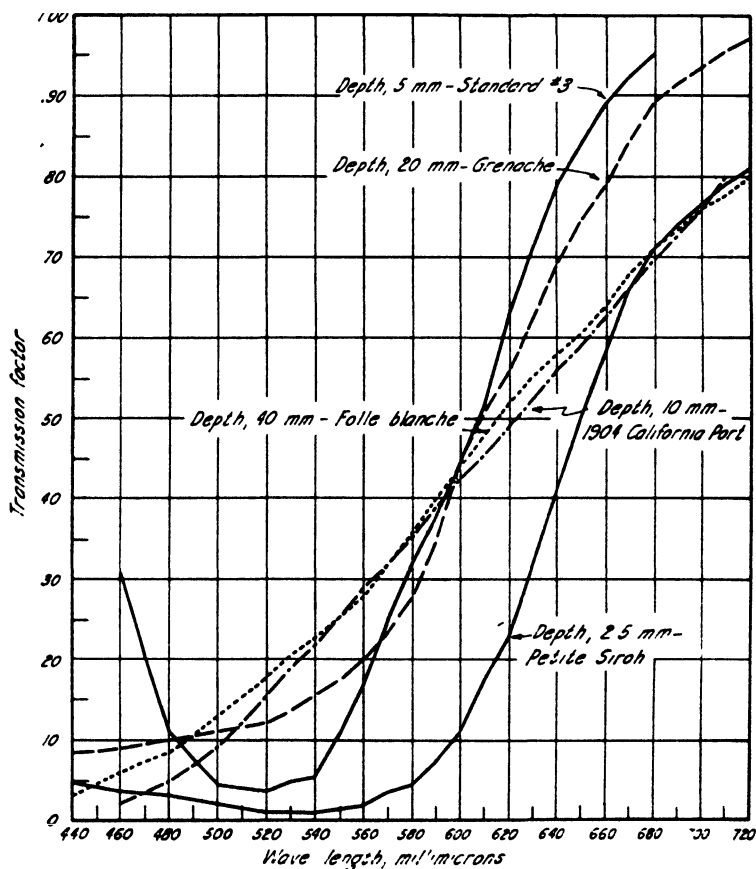


FIG. 1. Transmission curves of red and white wines and a color standard from 440 m μ to 720 m μ .

matic coefficients, brightness, purity, and dominant wave-lengths for the same samples (Table 1). In view of the great difference in brightness between red and white wines and the consequent absorption in red wines when too great a thickness is used, we have varied the depth for red and white wines.

The significant differences in the transmission curve between the various wines are apparent (Fig. 1). The curves are plots of the

TABLE 1
Color Data for Typical Red and White Wines and Some Color Standards as
Determined by Spectrophotometer Measurements

Variety or type	Approximate color	Trichromatic coefficients			Dominant wave-length	Depth	Purity	Brightness
		\bar{x}	\bar{y}	\bar{z}				
RED WINES								
Alicante Bouschet.....	Medium violet-red	.635	.309		504c ¹	mm.	pet.	pet.
Grenache.....	Pink	.585	.380		600	20	70	31.1
Mataro.....	Light red	.602	.348		619	5	64	21.5
Muscat Hamburg.....	Light red	.615	.344		616	5	71	15.4
Petite Sirah (1936).....	Dark red	.720	.280		648	5	99	1.6
Petite Sirah (1935).....	Dark red	.649	.316		655 ²	2½	73	9.6
1936 California port.....	Orange	.571	.401		595	5	83	39.6
1904 California port.....	Amber	.565	.421		590	10	94	56.0
Vergennes (fortified).....	Light pink	.531	.412		592	5	61	43.9
Grenache (fortified).....	Light pink	.520	.410		592	40	52	57.3
Grignolino.....	Very light pink	.565	.408		593	40	78	34.1
WHITE WINES								
Burger.....		.502	.424		587	40	49	56.6
Mixed whites.....		.550	.417		590	30	72	38.8
1932 California Gutedel.....		.518	.426		587	40	63	56.5
COLOR STANDARDS								
Alizarinastraviolet.....		.448	.367		570c	5	14	48.0
Croceine scarlet.....		.625	.373		600	5	100	45.0
No. 3 Standard.....		.601	.356		609	5	72	30.3
No. 3 Standard (diluted 1 + 1 with water).....		.605	.354		610	10	72	31.1
No. 3 Standard.....		.674	.326		613	10	99	15.7
No. 3 Standard.....		.690	.310		620	15	99	9.2

¹ The dominant wave-length of purple colors is not expressible in terms of any real spectrum color, but graphically may be expressed in terms of a complementary color. The terminal "c" indicates the complementary color of the wine. ² Between 650 and 680 millimicrons the graphical determination of dominant wave-length is only approximate.

data for measurements made every 10 millimicrons and no attempt has been made to smooth the curves.

Although we obviously do not have data concerning the original color of the 1904 California port, we have been assured that it was a red wine. The color at present is a medium amber, quite similar to certain sherries, and the lower dominant wave-length indicates that with time the red color has disappeared. Apparently the 1926 California port has also lost a considerable amount of its color with time. On the contrary, color of the young wines resembles that of the grapes from which they were made. The light-colored varieties have less blue than the darker-colored Petite Sirah and Alicante Bouschet. Since we have not completely standardized the depths at which the transmission factors were measured, attention is called to the fact that this has an important influence on the color data. Thus, if we had measured the white wines at a depth of only five mm., the transmission of light would have been so very great that measurement of the transmission factors would not have been so accurate but the brightness would have been much greater. The brightness of red and white wines are thus not strictly comparable; however, for these wines the data (Table 1) constitute a complete specification of the nature of their color for the conditions under which the measurements were made. This method, however, is tedious and since it involves the use of equipment which is rarely available in wineries, other means for comparison of the color of wine samples must be used. When a complete specification of the color of a wine is desired, it will require the spectrophotometer or a similar measuring device.

USE OF VINO-COLORIMETER AND COLOR-COMPARATOR

The Salleron-Dujardin vino-colorimeter has usually been employed whenever the color of wine has been measured in California wineries. This device is designed to measure both hue and brightness of the color. For the determination of hue, approximately the dominant wave-length, a scale is furnished consisting of a piece of cardboard on which are gummed small disks of silk which represent the approximate range of hue found in red wines. At the top of the scale is a disk marked VR (violet red) which is supposed to represent the hue of a new wine made from a well-colored grape of good acidity, such as Petite Sirah or Cabernet Sauvignon. At the bottom of the scale is a disk marked 3R (third red), the hue of wine from an inferior colored grape, probably with low acidity, such as Mission or Grenache under interior valley conditions in California. Between these extremes are eight other disks representing the intermediate hues usually found in red wines. Theoretically it is probable that if

a close examination of these disks were made by means of reflection measurements it would be found that they represented a range of dominant wave-lengths in the red because of a greater or less admixture with yellow and some blue. It is stated by the makers that all of the disks have been made of the same intensity (brightness) of color. To compare brightness of different wines the wine receptacle is placed opposite to a color disk and the color stimulus of the wine is equalized with that of the disk by screwing the cap of the receptacle up or down until an equivalent brightness is reached. Then the scale must be moved back and forth until the exact shade of disk is found. The hue of the wine is given by the number of the disk while the brightness of the sample is obtained from the scale reading (depth for equalization of brightness). The instrument reads brightness directly; that is, the depth of the sample is greater with increased transmissibility of light and vice versa. Hilgard (1887), however, suggested that the actual scale reading be divided into 4,000 so that the figures would be more nearly proportional to the color value of a wine. Then an intensely colored wine would have a high reading and a pale wine a low reading. Although this has been the usual procedure in recording color values in wineries, we shall record the readings as observed.

This method has at least three serious imperfections. First, the instrument is crudely made, hence its degree of precision is not very great. In intensely colored wines errors of 10 to 25 per cent may be introduced by pushing the cap up or down without turning it. Where many samples are handled and the operator does not handle the cap gently and in the same manner each time, measurable errors are introduced. Second, correct illumination is impossible without inclosing the device. Although means are furnished for partial inclosure of the instrument, use of the light shield interferes with its operation and ordinarily the shield is not used. Third, the color discs are not of uniform brightness (intensity) owing partially to the process of manufacture and also to the varying permanency of different hues.

Despite the limitations of the Salleron-Dujardin vino-colorimeter we have found its use to be expedient, since it enabled us to obtain approximate color values on a very large number of samples and to compare data secured by this and other means with earlier measurements on color of California wines obtained with this instrument.

The color-comparator or Duboscq colorimeter by which the brightness of an unknown is compared with that of a standard has not been used for wines in the past because of lack of suitable and permanent standard solutions. Mixtures of permanganate and bichromate were commonly used, but the colors were unstable and fresh

solutions had to be made up at frequent intervals. Vogt (1935) has now discovered several dyestuffs suitable for the comparison of wines and which he claims with considerable justification, to be stable. For normal German red wines he reports that Croceine Scarlet and Alizarinastroviolet in the ratio 35:65 milligrams per liter in distilled water give a good comparison. This solution showed no loss or change in color after standing in his laboratory for a period of a year. In our own work it has been necessary to double the concentration of the dyes to make them comparable with the average brightness of young California wines. Solutions standing in our laboratory

TABLE 2

Color Values of Dilution Tests of Wines as Determined With the Color-Comparator and the Vino-Colorimeter¹

Variety	Original color of wine		Color of wine diluted 1:1 with distilled water			
	Com-parator	Vino-color-imeter	Com-parator	Calcu-lated value	Vino-color-imeter	Calcu-lated value
Carignane.....	113	333	57	56.5	800	666
Petite Sirah.....	710	80	345	355	138	160
Cabernet Sauvignon.....	258	114	126	129	333	228
California port.....	50	666	25	25	1428	1332
Zinfandel.....	385	154	192	192	275	308

¹ A similar series of comparisons is given in Table 3 for some blending tests.

have remained stable as compared with newly prepared solutions after one year.

The transmission curve of a standard solution prepared with the two dyes was determined on the spectrophotometer and is shown (Fig. 1). Above 500 millimicrons it follows the transmission curve of a red wine quite well. The color characteristics of the standard are quite similar to that of a medium red wine, for example, the Mataro (Table 1).

The results obtained with the color-comparator, using the standard solution and comparative tests with the Salleron-Dujardin vino-colorimeter, are given (Table 2). The standard has been called 100 in this series of comparisons. The effect of dilution on the color values is also given.

These measurements indicate that the color-comparator is a more reliable means of measuring the brightness of wines than the vino-colorimeter. By means of the color-comparator approximate dilution and blending can be made and checked, while rather large deviations from the calculated values are obtained on the vino-colorimeter. These deviations could not be the result of discrepancies between operators

since all the measurements were made by one person under well-controlled conditions of operation.

The feature of the vino-colorimeter that has appealed to the wine maker is the arrangement by which both hue and intensity of color may be measured. In preliminary tests it has been found possible to mix different proportions of the two dyes and arrange a series from violet-red to orange. These tests have shown that solutions so prepared are approximately as satisfactory as the disks for determina-

TABLE 3
Color Values of Blending Tests of Wines as Determined With the Color-Comparator and the Vino-Colorimeter

Variety and blend		With color-comparator			With vino-colorimeter		
		Original color value	Color value of blend	Calculated value	Original color value	Color value of blend	Calculated value
Petite Sirah	1.....	710	500	510	80	140	120
Tannat	1.....	310			160		
Carignane	1.....	113	405	411	320	148	200
Petite Sirah	1.....	710			80		
Carignane	2.....	113	290	286	320	210	197
Zinfandel	1.....	210			170		
Petite Sirah	1.....	710			80		
Tannat	1.....	310	213	211	160	220	240
Carignane	1.....	113			320		
Petite Sirah	1.....	710	470	460	80	107	125
Zinfandel	1.....	210			170		

tion of the hue of a wine. Such solutions are less easy to manipulate than the disks but it is hoped that satisfactory instruments may be developed for their use. Their present inconvenience is partially compensated for by the greater permanence of the solutions.

The use of Lovibond slides has found wide application in commercial operations, especially in the textile industries where an approximate and quick standardization of color is needed. The color of a certain thickness of wine is matched with colored slides introduced into the instrument. The slides are made in blue, yellow, and red colors of different degrees of brightness. The number of units of slides indicates the approximate brightness of a wine. This is true where the red color greatly predominates but may be somewhat inaccurate for other combinations. The number of different slides used also indicates the approximate hue of the sample. One of the chief difficulties for accurate work has been found by the National Bureau

of Standards to be the lack of complete interchangeability of the slides. One of the uses for which the instrument may be well adapted is that of following the color change which occurs in a given wine with aging. We have made some preliminary observations of this nature. In the main the changes have not been large but have indicated the decrease in red and the increase in yellow with age. Complete data are given (Table 4).

We are indebted to the California Packing Corporation for the use of their extensive series of slides in this experiment. We have

TABLE 4
*Color Measurements of Various Wines With Lovibond Slides
on February 3, and May 5, 1937*

Variety	Depth of sample	Color value and composition					
		February 3, 1937			May 5, 1937		
		Red	Yellow	Blue	Red	Yellow	Blue
Burger.....	<i>inch</i> 1	1.5	2.7	0	.9	2.5	0
Zinfandel.....	1/16	15.6	0	0	14.7	0.2	0.5
Tannat.....	1/16	12.1	1.0	0.7	12.0	1.0	0.8
Carignane.....	1/16	4.0	1.6	0	4.0	1.6	0
Petite Sirah.....	1/16	28.5	0	0.9	27.0	2.0	0.5
Grenache ¹	1	2.1	1.8	0	2.5	2.4	0
MA4 ²	1/16	3.2	1.6	0	3.5	2.0	0
Standard No. 2.....	1/16	5.5	0.4	0.3	5.4	0.7	0.2
Standard No. 3.....	1/16	4.7	0.4	0.6	5.0	0.5	0.5

¹ Pressed immediately. ² Blend.

still to investigate special slides which have been manufactured by the makers of Lovibond slides for use on wines.

SUMMARY

1. Various methods for the measurement of color of wines have been investigated.

2. The spectrophotometric data may be calculated in terms which give a complete color specification for the wine.

3. The use of the term brilliance in wines is distinguished from brightness.

4. The Dujardin-Salleron vino-colorimeter gives only an approximate measure of two of the color characteristics of a wine.

5. The color-comparator is a fairly satisfactory means for measuring brightness when certain standards are used and it may be useful for measuring dominant wave-lengths when a satisfactory instrument for use of the standards is developed.

6. The Lovibond slides are useful for measuring changes in wines but are difficult to interpret for comparative values.

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COLOR IN CALIFORNIA WINES

II. PRELIMINARY COMPARISONS OF CERTAIN FACTORS INFLUENCING COLOR

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Commercially, color is one of the most important properties of a wine. Information concerning the factors which influence color and the means of their control is of considerable practical value. Wines made of different varieties of grapes produced under various environmental conditions may vary in color from a light straw-yellow, green-tinted wine through dark yellow or amber to light pink and finally to dark red, violet-tinted wines. Further, the original color may be modified by aging or other special treatment. Not only is the particular hue of a wine an important variable, but the brightness also will modify its beauty. These fluctuations in color are largely responsible for the initial, and sometimes the most important, impression that a wine makes on the observer.

Where wine types are adequately standardized the color of a particular type of wine varies only within the range of the varietal-regional limits which are established by differences in seasons. This standardization of color of wine types is made possible in the older viticultural areas of the world, in spite of their great changes in climatic conditions from year to year, largely because their varieties are restricted and the behavior of these few varieties with respect to different methods of vinification is well known. This does not mean that all the wines of a given district will have the same depth of color every year. On the contrary, in order to preserve the flavor and quality of a certain wine made in a poor season the wine maker may refuse to blend his wine with darker and poorer wines and thus decrease his quality; for example, the color of a Pinot wine of Burgundy may not always be the dark rich shade which we associate with the better quality of Burgundies, owing to the refusal of the wine maker to blend light-colored Pinot with the harsh dark Teinturiers.

In less-developed viticultural districts there is a lack of knowledge of the exact color behavior of different varieties not only with respect to locations but also effect of time in storage and cellar treatment of the wines. A widespread belief is prevalent that certain varieties do not retain their color under storage conditions. There is meager information on the effect of such treatments as pasteurization, freezing,

and fining on the stability of color of wines. Many opinions relating to the color instability of particular varieties may not only be traced to the peculiar composition of the color pigments of the wine but also to widespread incorrect use of various cellar treatments.

Despite such lack of definite information, the wine makers have recognized the great value of color and have put forth considerable effort to secure wines of a particular brightness and dominant wavelength. The first approach to securing of a desired color was to use varieties that most nearly matched the ideal or standard. Under winery conditions in California it is usually difficult to ferment the varieties separately, and only the broad limits of color value of different varieties are known. Owing to the wide color range of varieties, their inevitable mixing under California winery conditions and the lack of understanding of effects of different cellar treatments, particularly the important chemical changes taking place over long-time periods and their effects on color, it is difficult to attain sufficient color standardization. This is especially true of red wines, but white wines also are subject to color changes, primarily their natural tendency to darken. The color of white wines, however, may be partially protected from such undesirable changes by the judicious use of sulfur dioxide, or better by restricting exposure of the wine to air. The latter procedure, along with the use of the Reisling variety, is largely responsible for maintenance of the delicate green-tinted wines of the Moselle of Germany.

In making red wines the wine maker may not only utilize different varieties but he may alter the vinification to obtain wines of different brightness. The coloring matter of most red grapes is restricted to the cells of the skins. At the time of crushing the must is practically white and as the fermentation proceeds, more and more color is extracted from the skins. By drawing the wine off at various stages of fermentation the wine maker may vary the color from very light to the full degree of color of the grape itself, except, of course, with varieties the juice of which is colored and will yield considerable color no matter when the pressing is done. Further, heating the crushed grapes facilitates extraction of color so that the period of time before pressing may be reduced.

It is recognized that the above methods have to do with the extraction of color, but much of the color attraction of certain wines lies in the fact that the color is enhanced by brightness of the liquid. Nessler (1930) reports that the addition of tannin will increase the brightness of color of a red wine. Vogt (1932) has demonstrated a reduction in brightness of wines accompanying the addition of alkaline salts, although the color content was said to remain unchanged.

We have found a change in brightness by changing the pH of wines. Typical examples of the effect of change of pH on color are shown (Fig. 1 and Tables 1 and 2).

EXPERIMENTAL WORK

The data (Table 1) were obtained by use of the spectrophotometer on a 1917 California Burgundy. The wine was diluted 1 to 1 with

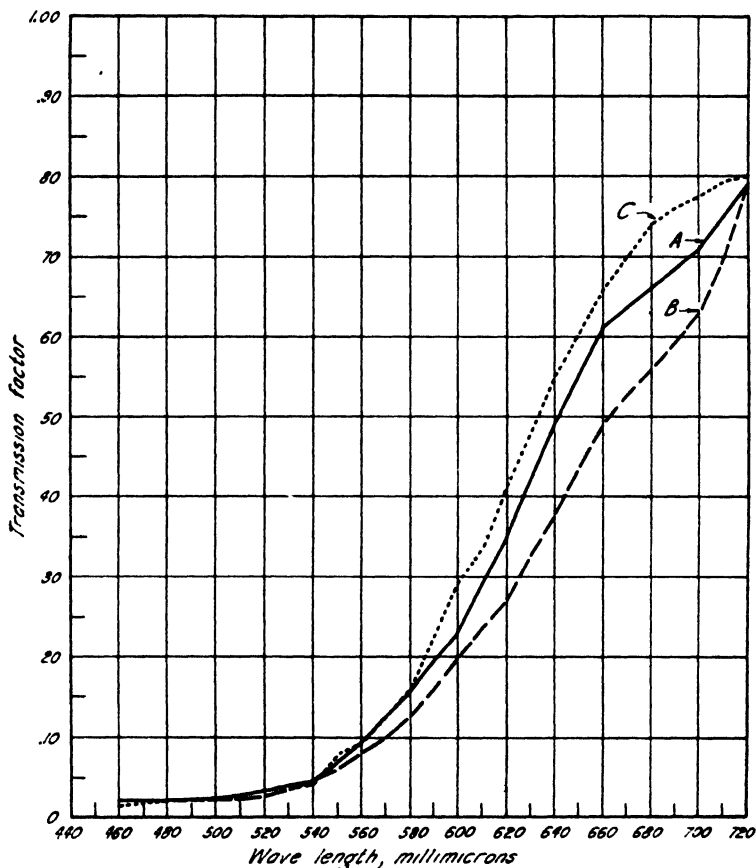


FIG. 1. Effect of pH on the transmission curve of a red wine from 460 m μ to 720 m μ .

water, H^+ , and OH^- ; and the table shows that as the pH is raised the brightness decreases, and as the pH is lowered the liquid becomes brighter compared with its normal condition. This means that the hydrogen ion affects the color pigments of the wine much the same as on any acid-base indicator. The transmission curves for the samples at different pH values are given (Fig. 1) and the color values as obtained with the spectrophotometer (Table 1). It is of interest

to note that not only is the transmission of light increased at the lower pH value, but also the purity of saturation of color of the wave-length 602 is increased. Similar spectrophotometric comparisons of the effect of changes in pH on the transmission curves of wine have been published by Montequi (1933) and Boutaric, Ferré, and Roy (1937).

TABLE 1
Color Data for Red Wine Diluted to Different pH Values as Determined by Spectrophotometer Measurements

Variety or type	Approximate color	Trichromatic coefficients		Dominant wave-length	Depth	Purity	Brightness
		\bar{x}	\bar{y}				
1917 California Burgundy	Medium red	.626	.364	604	mm. 5	pct. 92	pct. 17.2 ¹
1917 California Burgundy	Medium red	.623	.368	602	5	93	13.9 ²
1917 California Burgundy	Medium red	.632	.361	602	5	97	19.1 ³

¹ pH = 3.5 ² pH = 4.2. ³ pH = 2.1.

Changing the pH in commercial wine making has little to offer as a means of affecting brightness because of the large buffer capacity of the must and wine, except in so far as the maturity of the fruit itself affects the pH. Still uninvestigated, however, is the effect of pH on the extraction of color during the vinification. This point is receive-

TABLE 2
Effect of a Change in pH on Brightness of a Wine

Wine and treatments	pH	Brightness of color determined with color-comparator
Original wine.....	3.86	400 ¹
Original wine diluted 1:1 with water.....	3.81	205
Original wine buffered and diluted 1:1.....	1.45	330
Original wine buffered and diluted 1:1.....	2.45	250
Original wine buffered and diluted 1:1.....	3.42	200
Original wine buffered and diluted 1:1.....	4.13	175
Original wine buffered and diluted 1:1.....	5.12	161

¹ The brightness of the color standard was taken as 100.

ing consideration and will be reported on in another paper. Also, there is some confusion in the literature as to the effect of tannin, sulfur dioxide, and other treatments not only on brightness of wine but also on extraction of color from skins of the grapes.

In further studies it was deemed advisable to use for comparison not the color of the wine but the amount of color that could be extracted from the fresh grapes. The method was standardized so that

100 grams of grapes were thoroughly crushed and then heated in a beaker in a water bath at 26.7°C. (80°F.) for 45 minutes. After pressing, the colored juice was measured with the color-comparator or the Salleron-Dujardin vino-colorimeter. The data so obtained represent practically the maximum color which can be extracted from the grapes used.

The effort on the part of the wine maker to extract a large amount of color from certain varieties is sometimes unsuccessful owing to the fluctuation of color in varieties, depending on their inherent differences in amount of pigmentation as well as maturity of the grapes. The color values of typical varieties given are averages for California conditions and, although not completely independent of regional influences, they approximate the relative color content of the varieties (Table 3).

TABLE 3
Relative Color Values of Different Varieties

Variety	Color by vino-colorimeter
Grignolino.....	535
Aramon.....	495
Grenache.....	246
Carignane.....	73
Cabernet Sauvignon.....	51
Valdepeñas.....	44
Petite Sirah.....	37
Alicante Bouschet.....	27
Salvador.....	8

As shown in the table, regardless of what the wine maker may do he cannot obtain an intensely colored wine when using only such varieties as Grignolino or Aramon. On the other hand, a well-colored wine can be made of such varieties as Petite Sirah without the use of special methods for extraction of color, especially if the grapes are grown in a favorable environment and are picked at the proper stage of maturity for dry wines.

Not only are there great differences in the color values of the varieties but the color value in a given variety varies with the maturity of the fruit. The influence of maturity of the grapes on the color of wines six months after fermentation is shown (Table 4). Carignane and Alicante Bouschet were picked at three stages of maturity from the regions Delano, Merced, Davis, and the Santa Clara Valley.

The color values shown, however, are not strictly comparable since color values of the wines and not of the grapes are reported. At the

third stage of maturity there was a higher alcohol content at the end of the fermentation which materially aided in the extraction of color from the skins. Nevertheless, in the range of maturity at which grapes are usually picked for dry wines there is a wide variation in color content. This variation in pigment development is shown (Table 5) with the color values of must of Zinfandel grapes at different degrees of maturity as indicated by the degrees Balling of the juice. The same heating and pressing procedures were employed as previously described.

TABLE 4
Influence of Fruit Maturity on Color of Wines at Six Months

Alicante Bouschet			Carignane		
Degrees Balling	Color value		Degrees Balling	Color value	
	By color-comparator	By vino-colorimeter		By color-comparator	By vino-colorimeter
20.4	162	250	20.5	82	444
21.9	285	144	22.4	115	333
24.0	466	108	25.1	163	235

The differences in color content owing to variety and maturity (Tables 3, 4, and 5) are further complicated by the influences of environment, especially temperature. California offers a good opportunity for the study of this problem since there is a wide range of climatic conditions under which grapes are commonly grown. Since an adequate comparison of many varieties cannot be made for all regions, only the more common regions and varieties are given (Table 6).

TABLE 5
Influence of Maturity on Color Value of Zinfandel Grapes

Degrees Balling	Color value by vino-colorimeter
19.4	150
22.7	111
24.9	85
26.2	75

In each variety the color of grapes from the coolest growing region (of the least summation of heat) was twice to several times greater than that of grapes from the warmest growing regions (of the greatest summation of heat). This difference was independent of any difference in maturity, since the grapes used for comparison of any one variety are at approximately the same stage of maturity. This type of difference in color is well recognized by the growers of table grapes who are interested in securing a certain color in their grapes

for market. The difficulty in interpreting differences such as shown (Table 6) lies in determining the effect of seasonal heat on the color of grapes as compared with heat received during the ripening period. The authors have shown in another paper, Winkler and Amerine (1937), that the greatest differences between the regions under consideration is not in the total summation of heat for the growing period but mainly in the heat received during the ripening period.

That the temperature relation is a direct and important one is further strengthened by a comparison of the differences in color between two seasons, one known to have been well above an average temperature and the other an almost normal season. This influence of season is illustrated (Table 7) with the color values measured

TABLE 6
Influence of Regional Conditions on Color of Grapes

Variety	Regions and averaged color value (by vino-colorimeter)				
	Delano, Fresno	Lodi, Guasti, Davis	Livermore Valley, Asti, Ukiah	Napa Valley, Santa Clara Valley	South Sonoma Co., Santa Cruz Mts.
Alicante Bouschet.....	54	47	37	28	17
Carignane.....	89	82	70	48	40
Mattaro.....	500	286	200	73	62
Petite Sirah.....	57	50	45	28	20
Zinfandel.....	147	91	75	65	20

four months after fermentation for the same varieties from the same locations in 1935 and 1936.

Although the 1936 wines have a lower color value in each case, the difference between the two seasons was partially obscured, especially in the Petite Sirah wines of 1936, since this variety raisined very badly in each region and the higher sugar content of the fermenting must led to a higher alcohol content and thus gave a more complete extraction of color in 1936 than in 1935.

The data given (Tables 1 to 7) show the principal sources of variation in the color value of grapes and wines but by no means the only ones which the wine maker, especially of red wines, has to cope with. This material partially indicates the difficulties of obtaining attractive and uniformly colored wines of a given type from year to year. These difficulties are greater in a viticultural area such as California where many wineries at present try to produce nearly every "type" of wine.

In the small winery, where wines are produced from only a limited number of varieties grown in the immediate vicinity and where

only a few types of wine are made for the market, the wine maker can usually approach a uniformly colored product from season to season. The number of variations in color with which he works is relatively limited so that he soon becomes familiar with the colors produced by the possible blends and can readily reproduce them.

In the large winery, on the contrary, these difficulties are multiplied by (1) the many tanks of different wines, (2) the mixing of varieties during fermentation, (3) the use of grapes grown in several regions and mixing of them during crushing, (4) the large number of types of wines produced, and (5) the variation needed in color of the same type of wine to meet the demands of special markets. Under conditions such as these it is beyond the capacity of the wine maker's eye to determine components of the blend which will produce

TABLE 7
Influence of Seasonal Conditions on Color

Variety	Region, season, and color value (by vino-colorimeter)							
	Delano-Fresno		Lodi		Livermore		Napa	
	1935	1936	1935	1936	1936	1936	1935	1936
Alicante Bouschet.....	160	268	70	114	50	70	40	100
Carignane.....	200	400	182	362	66	102	73	105
Petite Sirah.....	100	268	80	134	36	50	31	40

the desired shade of color along with proper tannin, body, and flavor. The stock wines are so numerous and are of such a variable hue, purity, and brightness, together with considerable variation in body, flavor, aroma, and soundness, that there is an inevitable lack of uniformity of products and a consequent loss of quality.

It may be mentioned that in the production of port this same problem is further intensified by the restricted period of fermentation that these wines undergo which considerably reduces the amount of color that can be extracted from the skins.

The color problem with red wines in many wineries thus resolves itself into obtaining more accurate information concerning behavior of the color of grapes from different regions, at different stages of maturity, from different varieties, and between warm and cool seasons. Still unreported are the difference in stability of color in wines made from different varieties, and also the effect of various chemical conditions and treatments on the stability of these colors. Also under investigation are the effects of various field practices, such as irrigation, long and short pruning, etc., on the color of grapes produced.

SUMMARY

The importance of color in wine, the factors influencing its extraction in red wines, and the means of guarding against the undue darkening of the color of white wines are indicated.

Some of the primary sources of variation in the color value, especially of red wines, such as the variety, maturity of the fruit, regional conditions, and seasonal temperature, with supporting data are discussed. In this connection the problem of color standardization in the small winery as contrasted with that of the large winery is pointed out.

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SOURCES OF LEAD IN MAPLE SYRUP AND A METHOD FOR ITS REMOVAL¹

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The presence of heavy metals in foods, especially lead, and their effects upon the human body have been receiving considerable attention during the past few years. Only recently has lead been detected in some maple products. That these foods should have gone unsuspected is only reasonable, for they are produced from the pure sap of the maple tree which contains no lead. Any lead found in maple syrup, then, must have been introduced at some time during processing. This introduction of lead is wholly unintentional, and at no time during the production is it necessary for lead in any form to be brought into contact with either maple sap or syrup. To trace the source of this lead, it is necessary to consider carefully the entire process in its production. The sap is gathered from the maple trees in buckets. The sap buckets are made of a variety of materials, namely wood and iron plated with tin, terne (leaded tin), or zinc.

The oldest type of sap bucket undoubtedly is the wooden one, many of which are still in use. These wooden buckets, almost without exception, have been painted to protect them from weathering. The earliest tin-plated buckets were so heavily coated with tin that they are for the most part in excellent condition to-day. Later, however, only a very thin tin plate was used, so that after a few seasons the iron upon which the tin was plated began to strike through and show up as rust spots. Painting was resorted to in order to keep these buckets from rusting. Unfortunately no thought was given to the composition of the paint used on either the wooden or tin-plated buckets and, in most instances, it was one of high lead content. Another bucket-plating material that was found to be very resistant to weathering was an alloy of lead and tin known as terne plate. These terne-plated buckets were very popular since they weathered well and were cheaper than the better grade buckets plated with tin. The lead-bearing paint and the lead and tin alloy thus constituted the chief sources of lead contamination in maple syrup. Another minor source of lead is lead solder.

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During the sap-running season there is great variation in temperature, and especially toward the end of the season there is often enough warm weather to cause the sap, which contains from one and one-half to three per cent sucrose, to sour. This souring is caused by acid fermentation, and the acid so formed readily dissolves the lead from the coating of the bucket with which it comes in contact.

The ideal way to produce lead-free maple syrup would be to use only equipment the surfaces of which contain no lead. An extensive program is being carried on in the maple-producing areas of this country and Canada to bring this about, but it cannot be quickly accomplished. For the present the lead content of maple syrup must be reduced by removal of the lead, as is being done in the larger plants.

Maple syrup is rather distinct in that it is about the only food product of which the farmer is the producer, processor, and often the retailer. It is not practical or in many cases possible for the farmer to use the same deleading methods as are used in the large plants.

A simple and easy method which can be employed by the farmer-producer for the deleading of his maple syrup consists of using milk, a wholesome food, as the deleading agent. The lead of the syrup combines with the milk to yield a precipitate. The lead is precipitated with the curd of the milk which is formed when the mixture of the syrup and milk is heated to the boiling temperature. Almost without exception maple producers are also milk producers, so the milk for deleading of the syrup is readily available. Since it is the milk, exclusive of the butterfat, that forms the curd, either whole, skimmed, or dried milk may be used. The milk curd containing the insoluble lead can be removed from the syrup by filtration through cloth alone. Because the curd formed from this heated mixture is gelatinous, and because some of the fine particles of lead precipitate may pass through the cloth, better results are obtained by use of a filter aid. The milk is added to either hot or cold syrup and the mixture boiled for five minutes or until the water added as milk, has been removed. The mixture is then allowed to cool to about 65.6°C.(150°F.); the bulk of the milk curd formed is removed by skimming and the remainder by filtering.

A study was made to compare the effectiveness, as a deleading agent, of varying amounts of whole, skimmed, and dried milk with and without a filter aid. The original syrup before treatment with the milk contained 2.4 parts per million of lead. To aliquots of this syrup were added 5, 10, and 15 per cent by weight (Table 1) of whole milk. This was repeated with skimmed milk. In the case of

dried skimmed milk, amounts equivalent to the solids in 5, 10, and 15 per cent of skimmed milk were added.

The addition of five per cent of either whole or skimmed milk reduced the lead content of the syrup to about one-half its original content. Ten per cent of milk reduced it to about one-fourth and 15 per cent to about one-eighth its original lead content. The dried skimmed milk, when added in the same proportions as the dry matter of the skimmed milk, reduced the lead to only about one-half its original value.

The deleading of maple syrup is not the solution to the problem, but, until such time as the syrup can be made in lead-free equipment, it is better to have it deleaded with milk, a wholesome food, than to

TABLE 1
Effectiveness of Milk as a Deleading Agent of Maple Syrup

	Per cent of milk by weight added to syrup					
	5		10		15	
	Filter aid	No filter aid	Filter aid	No filter aid	Filter aid	No filter aid
	p.p.m. of lead	p.p.m. of lead	p.p.m. of lead	p.p.m. of lead	p.p.m. of lead	p.p.m. of lead
Whole milk.....	1.14 ¹	1.36	0.90	0.98	.30	0.34
Skimmed milk.....	1.07	1.28	0.48	0.52	.32	0.22
Dried skimmed milk.....	1.40	1.78	1.05	1.28	.95	1.10

¹ The original syrup contained 2.40 p.p.m. of lead.

let it go to the consumer untreated. In all fairness to the consumer any maple products which have been processed with milk ought to be so labeled.

Fortunately the situation is not as bad as it might appear. Of 500 samples of maple syrup produced in New York state, in the customary manner and without thought of lead contamination, only 80 contained an amount of lead which would be considered significant.

SUMMARY

The chief sources of lead in maple syrup are (1) lead paint which has been used to paint maple-producing equipment and chiefly sap buckets; (2) lead tin alloy used as a coating over some iron sap buckets, spouts, evaporators, and storage tanks; (3) lead-tin solder used in making water-tight joints in the above-mentioned equipment. Lead is dissolved by the sap, under the right conditions of temperature and acidity, when it is in contact with these lead-bearing materials.

The syrup can be produced lead-free by modifying the equipment so as to eliminate lead-bearing materials or by painting with a lead-free paint.

If the lead has been allowed to dissolve in the syrup, it can be removed as a phosphate, a method which is practical only in plants handling large amounts of syrup. The smaller producer may delead (clarify) his syrup by the addition of ten per cent or more by weight of skimmed or whole milk, heating, and subsequent filtration.

FURTHER OBSERVATIONS ON PRODUCTION OF ALCOHOL BY *SACCHAROMYCES ELLIPSOIDEUS* IN SYRUPED FERMENTATIONS

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The effects of temperature, kind of juice, and method of increasing sugar content upon alcohol production by *Saccharomyces ellipsoideus* in syruped fermentation were reported previously by Hohl and Cruess (1936). In this paper are presented the results of further studies upon this method of producing wines of high alcohol content. The procedure used was substantially that described previously. The culture medium consisted of fresh grape juice or of grape concentrate diluted to approximately 22° Brix and sterilized in glass bottles of suitable size stoppered with cotton. Several strains of wine yeast were used. The fermentations were syruped repeatedly with grape concentrate each time the Brix degree decreased to between 0 and 5°, until fermentation ceased. The amount of grape concentrate added was such that the sugar content of the fermenting liquid was increased approximately five per cent at each syruping. After fermentation had ceased the wines were analyzed for alcohol and volatile acid content.

STATISTICAL STUDY OF SYRUPED FERMENTATIONS

During the course of these studies, frequently greater differences in the final alcohol and volatile acids have been observed between two duplicate samples than between the means of two or more pairs of samples which were to be compared. For this reason it was thought desirable to conduct a relatively large number of fermentations under identical conditions and statistically analyze the resulting alcohol and volatile acid contents in order to determine how many fermentations are required to obtain a reliable mean.

Twenty-five fermentations were conducted in pint grape-juice bottles, containing an initial volume of 200 c.c. of sterilized Thompson seedless grape juice inoculated with two per cent (by volume) of active Tokay yeast starter. The fermentations were syruped with grape concentrate on the fourth, sixth, eighth, eleventh, and twentieth days. When fermentation was complete, alcohol and volatile acidity were determined in duplicate. The results of these analyses are summarized and the means, extremes, and standard deviations are given (Table 1).

If, as in this case, 25 samples are used in a single series, the standard deviations or the limit of significance of the mean for the alcohols attained is .1 per cent. If this limit of significance is arbitrarily increased to .2 per cent, the number of fermentations to be made will be decreased as follows:

σ_M = Arbitrary standard deviation or limit of significance = .2

σ = Standard deviation of the series = .5

n = Number of samples

$$\sigma_M = \frac{\sigma}{n} \quad n = 6\frac{1}{4} \text{ or } 7 \text{ samples.}$$

Similarly if σ_M is arbitrarily increased to .3 per cent, n is decreased to three.

Therefore, if experimental fermentations are made in triplicate, the means must differ from each other by at least .3 per cent of alco-

TABLE 1
Statistical Study of 25 Syruped Fermentations

	Alcohol by volume	Volatile acidity (acetic per 100 c.c.)
	<i>pct.</i>	<i>gm.</i>
Number of samples analyzed.....	25	25
Maximum.....	17.9	.100
Minimum.....	15.9	.051
Average.....	16.7 \pm 0.1	.077 \pm .002
Standard deviation.....	0.5 \pm 0.07	.009 \pm .0013

hol in order to be significant. Since the error in alcohol determination may amount to .2 per cent, a safer procedure would be to make fermentations in triplicate and set a difference of .5 per cent alcohol as significant. If duplicates are used σ_M becomes .35 and a difference of .6 per cent between means of duplicates may be considered as significant.

EFFECT OF SYRUPING UPON YEAST CROP

The periodic addition of syrup to a fermenting wine has been found to result in a higher yield of alcohol than that attained in a medium to which the same total amount of sugar was added initially. This was believed to be related to the maintenance of yeast activity at a high level. Therefore a study of the effect of syruping on yeast crop was made.

For estimation of the yeast crop, direct microscopic counting in a haemocytometer, dilution plating on agar, and estimation of dry weight of yeast per unit volume were compared by a modification of

the method described by Balls and Brown (1925). Direct counting was abandoned because it was not possible to obtain consistent results. It was also objectionable because it did not differentiate between living and dead cells. Dilution on nutrient agar overcame this latter objection, but significant counts were difficult to make because of the strong tendency for the yeast cells to clump. Determination of dry weight could be criticized also because it does not distinguish between living and dead cells. Furthermore, autolysis, in the later stages of fermentation may introduce inaccuracies into results thus obtained. In these experiments, dilution plating and determinations of dry weight of yeast crop were made on the same samples.

Portions of two liters each of filtered and sterilized grape juice in gallon jugs were inoculated with an active yeast starter of yeast No. 66 (Series A). A like experiment was made with Tokay yeast (Series B). The yeasts are both non-agglomerating strains of *Saccharomyces ellipsoideus*. In each series the yeast was inoculated in duplicate into (a) juice of 20° Brix to which no syrup was added, i.e., normal or "straight" fermentations; (b) 20° Brix juice syruped in the usual manner, when the Brix degree fell below 5; and (c) 35° Brix juice, i.e., "straight" fermentations, which did not become "dry" (nearly sugar-free) as did those with initially 20° Brix.

For dry-weight determinations, acid-washed asbestos mats were prepared on Gooch crucibles, dried, and weighed. The fermenting juice was thoroughly shaken in order to secure as uniform a suspension as possible. Samples of 100 c.c. of the juice were taken, and each was mixed with one gram of infusorial earth to hasten filtration, filtered, washed, dried at 100°C. (212°F.), cooled in a desiccator, again weighed, and the weight of the yeast crop calculated.

For estimation of the viable crop, one c.c. of the yeast suspension was removed from the fermentation vessel with a sterile serological pipette and transferred to 99 c.c. of sterile tap water in a six-ounce medicine bottle. This was thoroughly shaken, and further dilution in sterile water was made as required. Experience in preliminary experiments made it possible to determine which dilutions were likely to be suitable. Plates having between 50 and 300 colonies were chosen for counting. Instead of Petri dishes, flat, six-ounce medicine bottles were used for plating and counting. Counts were made after three or four days of incubation at room temperature.

From the accompanying tables, it is evident that 35° Brix must be not as favorable a medium for the production of a large yeast crop (and incidentally, also not so suitable for alcohol production) as is a 20° Brix must, or as the syruped fermentation.

The syruped fermentation apparently produced a somewhat larger crop on the dry-weight basis and this tended to remain slightly above that of the control throughout the fermentation period (Tables 2 and 3). The viable cell counts, however, showed the opposite effect, that is, apparently a larger number of viable cells were produced in the controls than in the syruped fermentations. Nevertheless, yeast-crop estimation by direct weighing is to be regarded as more reliable than the plate-dilution count, because syruping causes the yeast cells to clump while the controls do not clump. As yet, no means of overcoming clumping after syruping has been devised. A number of

TABLE 2
Dry Weight of Yeast Crop and Number of Cells per Cubic Centimeter in Series A (Yeast No. 66)

Age of culture	20° Brix, not syruped		Syruped fermentation (started at 20° Brix)		35° Brix, not syruped	
	Dry weight	Number of cells per c.c. ($N \times 10^6$)	Dry weight	Number of cells per c.c. ($N \times 10^6$)	Dry weight	Number of cells per c.c. ($N \times 10^6$)
<i>days</i>	<i>gm.</i>		<i>gm.</i>		<i>gm.</i>	
0	.005	11.4	.008	12.5	.019	10.5
1	.076	60.0	.089	60.0	.018	7.4
2	.197	670.0	.242	810.0	.151	126.2
3	.261	1,002.5	.305	1,070.0	.167	447.5
4	.374	940.0	.391 ¹	1,005.0	.209	580.0
5	.425	1,227.5	.433	1,015.0	.219	355.0
8	.408	640.0	.453	515.0	.232	195.0
10	.381	441.2	.466	440.0	.282	165.0
13	.376	137.5	.460	130.5	.290	32.9
15	.316	61.7	.408	26.5	.262	25.0
17	.317	82.5	.395	29.5	.236	6.1
20	.293	14.7	.402	9.3	.230	0.8

¹ The underscorings represent the times at which syrup was added to the respective fermentations.

strains of yeast were compared with respect to this phenomenon, and while Tokay and No. 66 were found to clump the least, they still do so to a noticeable extent. Until this difficulty can be satisfactorily overcome, it will not be possible to get accurate crop estimates by the dilution-plating method. In all cases the number of viable cells tended to increase to a maximum in five to seven days and then rather rapidly to diminish.

Analyses of the fermented juices yielded the following results: Yeast No. 66 in unsyruped, 20° Brix must produced 13 per cent alcohol by volume and .086 gram of volatile acid (as acetic) per 100 c.c.; and the resulting wine had an extract content of 1.5° Brix. In syruped, 20° Brix must, 17.3 per cent of alcohol, .081 gram of acetic

acid, and 6° Brix of extract resulted. In unsyruped, 35° Brix must, 12.3 per cent of alcohol, .246 gram of acetic acid, and extract of 15.3 Brix were produced. Tokay yeast under similar conditions yielded the following respective results: 12.2 per cent alcohol, .063 gram acetic acid, 2.5° Brix; 15.4 per cent alcohol, .058 gram acetic acid, 7° Brix; 13.4 per cent alcohol, .189 gram acetic acid, 15.5° Brix.

EFFECT OF AERATION

A. *Effect of Depth of Liquid (Surface-Volume Ratio)*: During the course of previous experiments, it was noted that fermentation of

TABLE 3
Dry Weight of Yeast Crop and Number of Cells per Cubic Centimeter in Series B (Tokay Yeast)

Age of culture	20° Brix, not syruped		Syruped fermentation (started at 20° Brix)		35° Brix, not syruped	
	Dry weight	Number of cells per c.c. (N × 10 ⁶)	Dry weight	Number of cells per c.c. (N × 10 ⁶)	Dry weight	Number of cells per c.c. (N × 10 ⁶)
<i>days</i>	<i>gm.</i>		<i>gm.</i>		<i>gm.</i>	
0	.039	7.0	.032	8.1	.093 ¹	9.6
1	.203	82.0	.269	94.5	.169	83.0
2	.311	1,420.0	.388	1,315.0	.267	815.0
4	.402	1,080.0	.402	1,070.0	.293	379.0
5	.409	1,090.0	.435	1,015.0	.238	510.0
6	.417	1,050.0	.488	765.0	.236	412.0
7	.415	915.0	.436	730.0	.315	485.0
9	.360	795.0	.389	460.0	.300	302.0
11	.314	525.0	.410	275.5	102.5
13	.315	369.0	.376	201.0	.249	34.9
15	.271	205.0	.370	118.5	.259	10.7
18	.236	25.9	.344	42.0	.221	2.9
21	.256	3.4	.284	13.6	.288	1.5

¹ In this experiment the original must had more solid matter than the others, causing the original weight to be high.

large volumes of juice frequently gave higher alcohol yields than fermentation of small volumes. This observation suggested the possibility that the oxygen tension of the fermenting liquid might influence alcohol production, and that lower oxygen tensions had a more favorable effect. In order to test this hypothesis several experiments were projected, the first of which was to test the effect of surface-volume ratio upon the alcohol yield, using the syruped-fermentation method.

Six one-liter, graduated, cotton-stoppered, mixing cylinders were sterilized in dry air and 100-, 400-, and 700-c.c. portions of sterile 20° Brix grape must were placed in the respective cylinders in duplicate.

All the cylinders thus had equal surface areas but contained varying volumes of liquid. In order to extend the series to include still smaller surface-volume ratio, six five-foot lengths of three-cm. diameter Pyrex glass tubing were stoppered at the lower end with paraffin-coated rubber stoppers, sterilized chemically with 50 per cent alcohol containing 500 parts per million of SO_2 , rinsed with sterile distilled water, partially filled respectively with 200-, 400-, and 600-c.c. portions of sterile must, and stoppered with sterilized cotton plugs.

Each lot of juice was inoculated with two per cent by volume of active starter of the Tokay strain of yeast, and fermentation was allowed to proceed at room temperature. Syruping was conducted as usual.

It appears that there is a tendency for the alcohol yield to decrease with decrease in surface-volume ratio (Table 4). These results

TABLE 4
Effect of Surface-Volume Ratio Upon Alcohol and Volatile Acid

Initial surface-volume ratio	Final surface-volume ratio	Final alcohol content of wine by volume	Volatile acid (acetic per 100 c.c.)
		<i>per</i>	<i>gm</i>
1:3.3	1:4.5	16.4	.043
1:13.0	1:17.3	15.5	.031
1:22.8	1:33.0	15.4	.029
1:39.0	1:53.0	14.0	.027
1:73.0	1:88.0	13.0	.028
1:106.0	1:134.0	13.8	.031

are in agreement with those of Luers and Schmal (1927) who reported that in glass vats of different shapes the following comparative amounts of fermentation were observed—deep 56.2, shallow 58.9, shallow (with agitation) 61.6 per cent.

B. Effect of Agitating Juice During Fermentation: Two per cent of active starter of Tokay yeast was added to each of six 500-c.c. portions of grape juice of 20° Brix in cotton-stoppered, quart, grape-juice bottles. Three were thoroughly shaken once a day, while the other three were left undisturbed except when the addition of grape concentrate made shaking necessary. Each fermentation was syruped on the third, sixth, and fourteenth days, with an amount of grape concentrate sufficient to increase the total sugar content approximately 10 per cent.

The analyses after fermentation indicated that thorough shaking once a day lowered the final alcohol yield by about .5 to 1 per cent or an average of .7 per cent.

C. Effects of Oxygen, Nitrogen, and Carbon Dioxide: There is extensive literature concerning the effects of oxygen upon fermentation. Lafar (1907) has reviewed the early literature, Windisch (1933) and Hoogerheide (1935), the later. Reference will be made here only to those observations which have a direct bearing upon the present problem.

Pasteur (1876) was the first to observe that a larger crop of yeast and lower yield of alcohol were secured in the presence of air than in the absence of air. Lühder (1920) observed that fermentation in closed tubs produced slightly higher alcohol content than in open ones. Gorr and Perlmann (1926) in experiments with baker's yeast showed that from 20 grams of sucrose, 24.64 grams of alcohol were produced in a culture which for 60 hours was aerated with 715 liters of air; whereas in the control, non-aerated cultures 25.98 grams of alcohol appeared in 54 hours. Brewer's yeast, however, did not behave in the same manner. Bengtson (1932) showed that small additions of oxygen enhance the fermenting power of yeast, but large additions adversely affect it. Tomoda (1936) reported alcoholic fermentation to be depressed to some extent by aeration.

Hofmann (1930) in studying the influence of carbon dioxide on fermentation by yeast pointed out that the main difficulty with earlier experiments had been the use of high pressures, under which some other factors may also come into play. He concluded that there is no doubt that CO_2 dissolved in high concentrations inhibits fermentation.

The fermentations in this experiment were conducted in gallon jugs, each containing one and one-half liters of sterile grape juice of 20° Brix. Each gas was passed from a cylinder of the liquefied gas by means of a T tube through two sterile, cotton-filled, calcium-chloride tubes and then into the juice through inlet tubes with small openings which dispersed the gas in a stream of fine bubbles. On leaving the fermentation vessels, the gases were passed through 18 N sulfuric acid in absorption towers in order to prevent loss of alcohol from the system. The tests with each gas were made in duplicate in each of two separate experiments. In the first experiment a continuous flow of the gases through the media was used. In the second experiment the gases were bubbled through the media for one hour each day. In each experiment the media were saturated with the respective gases before inoculation with two per cent of active Tokay yeast starter. The final alcohol content in the absorption towers was determined by the method of Semichon and Flanzky (1929).

From the results (Table 5) it appears that carbon dioxide inhibited alcohol production, especially when it was continuously bubbled through the medium. Likewise, nitrogen did not allow the attainment

of maximum alcohol yields, although its effect was not so great as that of carbon dioxide. On the other hand, a continuous flow of oxygen through the media allowed more alcohol to be formed than in the controls, although at the expense of more additions of syrup. The latter were fermented in the normal manner in cotton-stoppered jugs. Brown and Balls (1925) have shown that there is considerable oxidation of alcohol into carbon dioxide under the influence of strong aeration. With a daily one-hour period of oxygenation, about the same amount of alcohol was produced as in the controls. There is a large difference between the alcohol content of the duplicates. A similar

TABLE 5
Effect of Gas Treatments on Syruped Fermentations

Gas	Treatment	Number of syrupings	Total alcohol ¹ by volume	Volatile acidity (acetic per 100 c.c.)	Aldehyde in wine
			<i>pct.</i>	<i>gm.</i>	<i>p.p.m.</i>
CO ₂	Continuous	0	9.1	.067	3
CO ₂	Intermittent ²	0	12.2	.115
N ₂	Continuous	2	13.7	.051	5
N ₂	Intermittent	1	12.7	.102
O ₂	Continuous	5	18.7	.068	26
O ₂	Intermittent	3	14.4 — 17.5 ²	.100
Control	(Of continuous series)	3	17.8	.063	18
Control	(Of intermittent series)	3	16.9	.084

¹ These values are corrected for the alcohol absorbed in the H₂SO₄ towers. The amounts of alcohol found in the traps varied from .7 to 3.8 grams. ² Gas bubbled through for one hour per day. ³ The individual results varied too much for a significant average. On repetition the same variation was found.

but smaller discrepancy, however, was found when this experiment was repeated: controls 17.8 and 18; oxygen 16.5 and 17.6 per cent.

After the samples from the first series had been stored for six months, aldehydes were determined by the iodimetric method of Kolthoff and Furman (1929). As was to be expected, the oxygenated fermentations contained the most aldehyde, while those with N₂ and CO₂ contained less than the controls.

Apparently as high an alcohol production may be obtained in presence of oxygen as in its absence if a sufficient amount of sugar is present. Since the periodic oxygenation of the medium resulted in no significant decrease in alcohol formed, the formation of larger amounts of alcohol from large-scale fermentations than from small-scale ones cannot be ascribed to the greater degree of aeration of the latter. It is possible that more alcohol is lost by evaporation from the

smaller samples. Pohl (1937) has shown that alcohol lost by evaporation during fermentation of molasses amounts to 1.22 per cent in closed vats and 1.95 per cent in open ones.

COMPARISON OF ALCOHOL-FORMING POWER OF SEVERAL STRAINS OF YEAST

Thirty-four strains of yeast, of which 32 were probably *Saccharomyces ellipsoideus* and two were commercial bread yeasts, were com-

TABLE 6
Effect of Yeast Strain

Yeast	Source	Alcohol by volume— mean of duplicates
		pct.
Burgundy.....	Fruit Products Laboratory	18.5
Burgundy, Tokay and Champagne (mixed).....	Fruit Products Laboratory	17.7
Champagne (a).....	Fruit Products Laboratory	17.0
Champagne (b).....	Laboratory class (1936)	17.2
Champagne (c).....	Laboratory class (1936)	17.3
Champagne (d).....	Laboratory class (1936)	17.2
Champagne (e).....	Wente Winery, Livermore, Calif. (1936)	16.4
Distiller's.....	Berkeley Yeast Laboratory	16.7
Loganberry (Canada).....	University of Washington, Seattle	18.1
Muscatel.....	Pasteur Institute, Paris, France	17.5
Muscatel (Geisenheim).....	University of Washington, Seattle	17.1
Port.....	Pasteur Institute, Paris, France	15.3
Raspberry.....	University of Washington, Seattle	16.1
Sherry.....	Pasteur Institute, Paris, France	17.2
Sherry.....	Spanish "Jerez"	17.1
Tokay.....	Fruit Products Laboratory	17.8
Wine (a).....	Frei Bros., Healdsburg, California	17.2
Wine (b).....	Frei Bros., Healdsburg, California	17.5
Wine (c).....	Frei Bros., Healdsburg, California	16.6
Wine (d).....	Italian Swiss Colony, Asti, California	17.8
Youngberry (2).....	Fruit Products Laboratory	17.2
317 (a).....	Tulare Winery, Tulare, California (1934)	16.4
320 (a).....	Santa Lucia Winery, Fresno, Calif. (1934)	17.2
351.....	Sulfited orange wine	18.0

pared in respect to maximum alcohol production in 150-c.c. portions of sterile must which were syruped during fermentation.

The yeasts varied considerably in respect to the final alcohol content attained, the lowest average being 10.3 produced by a yeast from Michigan Agricultural Experiment Station, and the highest average of two trials, 15.9, produced by Fruit Products Laboratory

yeast No. 351. Tokay yeast attained 15.1 per cent; Kingsburg, California wine yeast, 15 per cent; Geisenheim 1933 yeast, 14.9 per cent; and several others, 14 per cent or more.

In a second experiment several new strains of yeast were tested, and for comparison some of the strains previously tested were also included.

The procedure was essentially similar to that previously described, except that 200 c.c. of grape juice was fermented in pint grape-juice bottles instead of 300-c.c. Erlenmeyer flasks.

In this series of experiments the alcohol yields were all higher and the volatile acids lower than in the preceding experiment. The data show that the strains which gave the best results were Burgundy,

TABLE 7
Effect of Size of Inoculum

Yeast	Volume of starter	Alcohol	Volatile acid (acetic per 100 c c.)
	<i>pct.</i>	<i>pct.</i>	<i>gm.</i>
Tokay.....	1	18.1	.063
Tokay.....	2	18.2	.063
Tokay.....	4	17.6	.076
Tokay.....	7½	18.3	.091
Tokay.....	10	18.0	.076
Loganberry.....	1	18.2	.064
Loganberry.....	2	18.8	.073
Loganberry.....	4	18.2	.073
Loganberry.....	7½	19.2	.067
Loganberry.....	10	19.2	.061

351, Canadian loganberry yeast, Tokay, and the Italian Swiss Colony's (Asti, California) yeast (Table 6). This latter yeast was isolated from a fermentation which was active at 40°C.(104°F.), an unusually high temperature for commercial wine fermentations.

Many investigators, including Kayser (1924); Semichon (1929); Mezzadrolì, Amati, and Sgarzi (1931); Pique (1933); Ventre (1936); and Henry (1937), have pointed out that the use of various strains of *Saccharomyces ellipsoideus*, especially selected for each must to be fermented, increases the yield of alcohol, accelerates the rate of fermentation, and imparts a characteristic bouquet depending upon the strain used.

EFFECT OF SIZE OF INOCULUM

It was thought that the size of active yeast starter added to the must, might be a factor in determining the final alcohol attained. Syruped fermentations were conducted in the usual manner, except

that the amount of the yeast suspension used as a starter was varied from one to 10 per cent by volume. Two strains of yeast, Tokay and a Canadian loganberry yeast (received from the University of Washington), were used. The experiments were made in triplicate in two

TABLE 8
*Effect of Previous History of Yeast Starter
on Alcohol Production*

Starter	Brief description	Alcohol (mean of triplicates)
		<i>pct.</i>
A.....	Grape juice, 7 days old, from agar slant	16.7
B.....	Grape juice, 5 days old, from A	15.7
C.....	Grape juice, 3 days old, from B	16.4
D.....	Grape juice, 2 days old, from C	16.5
E.....	Grape juice, 1 day old, from D	16.2
F.....	Grape juice, 6 days old, from fermenting must	15.6
G.....	Grape juice, 5 days old, from F	16.1
H.....	Grape juice, 4 days old, from G	15.6
I.....	Grape juice, 3 days old, from H	15.9
J.....	Grape juice, 2 days old, from I	16.5
K.....	Grape juice, 1 day old, from J	16.7
L.....	Grape juice, 3 days old, kept at 32.2°C. (90°F.)	16.6
M.....	Grape juice, 2 days old, kept at 32.2°C. (90°F.)	16.8
N.....	Grape juice, 1 day old, kept at 32.2°C. (90°F.)	17.3
O.....	Grape-agar slant, 1 day old	16.1
P.....	Grape-agar slant, 3 days old	15.7
Q.....	Grape-agar slant, 4 days old	15.8
R.....	Grape-agar slant, 5 days old	16.8
S.....	Grape-agar slant, 3 weeks old, at room temperature	16.5
T.....	Grape-agar slant, 3 months old, at room temperature	16.6
U.....	Grape-agar slant, 8 months old, at 2.2°C. (36°F.)	16.7
V.....	Grape juice, 3 days old, from U	17.0
W.....	Grape juice, 3 days old, from V	16.3
X.....	Grape juice plus 2 per cent pure alcohol	16.6
Y.....	Grape juice plus 5 per cent pure alcohol	16.2
Z.....	Grape juice plus 10 per cent pure alcohol	17.3
A'.....	Grape juice plus 2 per cent wine alcohol	16.3
B'.....	Grape juice plus 5 per cent wine alcohol	16.6
C'.....	Grape juice plus 10 per cent wine alcohol	16.9

series with the mean results shown (Table 7). An examination of the data shows that no constant or predictable effect may be ascribed to the size of the inoculum employed. Only the data from one series are presented, since the second series gave very similar results. It was observed that with a one-per cent inoculum fermentation was rather slow in starting.

EFFECT OF PREVIOUS HISTORY OF YEAST STARTER

Starters of Tokay yeast were prepared as briefly indicated (Table 8). Zinfandel grape juice and Thompson seedless grape juice in 200-c.c. portions, previously sterilized in pint bottles, were inoculated with two per cent of the respective liquid cultures or with a loopful of the agar slant. The fermentations were syruiped in the usual manner. As will be seen from the table, no very consistent effect of method of storage or age of the starters used was observed; although previous growth of the starter in an alcoholic medium may favor increased alcohol formation.

SUMMARY

Further studies on high alcohol production by *Saccharomyces ellipsoideus* by syruiped fermentation are reported in this paper.

1. From a statistical study of 25 syruiped fermentations the conclusion was drawn that differences of .5 per cent alcohol by volume between the means of triplicate fermentations were significant.

2. The effect of syruiping upon the yeast crop was studied, using dry-weight and plate-counting data, and it was shown that the addition of syrup maintains the yeast crop at a slightly higher level than that held by the control fermentations without syrup additions. This may account in part for the greater alcohol production by syruiped fermentation and may indicate successive slight increases in yeast crop with syruipings.

3. Large-scale fermentations invariably resulted in higher yields of alcohol than those conducted on a small scale.

4. Alcohol production was found to decrease as surface to volume ratio of the fermenting liquid decreased. Daily agitation of the fermentation resulted in a lowered alcohol yield. Aeration of the fermentation with oxygen caused higher alcohol production and a higher sugar consumption in comparison with the control, whereas nitrogen decreased the production somewhat. Carbon dioxide decreased it greatly.

5. Nearly 50 strains of yeast, mostly wine yeasts, were compared and were found to differ considerably in their alcohol production in syruiped fermentation.

6. The size of inoculum (between one and 10 per cent by volume of starter) was found to exert little or no effect upon the final alcohol attained.

7. The previous treatment of the starter appears to have very little effect upon the alcohol attained.

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PRESERVATION OF GRAPE JUICE¹

V. PASTEURIZATION OF GRAPE AND APPLE JUICES FOR STORAGE OR IMMEDIATE FERMENTATION

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Preservation of fresh fruit juices by any heat treatment requires a careful study of the temperatures necessary for pasteurization or sterilization, since excessive heating may cause changes in the delicate flavors and aromas, and certain chemical constituents may be coagulated or otherwise changed. This is true of grape juices which are to be either fermented into wines at once or held in storage as the unfermented product to be fermented at a later date. In commercial practice it is possible that certain lots of grapes picked during adverse weather conditions may be delayed in reaching the winery or that they may not be used immediately after reaching the winery, with the result that the number of undesirable microorganisms may increase to such a point that they predominate in the fermentation. In preparing juices for fermentation into wines a majority of the microorganisms have to be killed in order that a pure-culture yeast inoculum may predominate; whereas in preparing juices for storage as the unfermented product it is necessary to kill all microorganisms capable of causing spoilage or fermentation.

The purpose of this study was to determine the lowest temperatures necessary to prepare fresh juices for immediate fermentation into wines or for storage as the unfermented product.

Studies on the pasteurization and preservation of flavor and quality in Concord grape juice have been reported in previous papers by Pederson (1936) and Tressler and Pederson (1936). Studies have also been reported on the open-kettle method of pasteurization of grape juices for fermentation or storage purposes by Pederson, Beavens, and Goresline (1936).

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EXPERIMENTAL PROCEDURE

Freshly pressed juices from Delaware, Elvira, Catawba, and Concord grapes and from Baldwin apples were heated at different temperatures in several types of pasteurizing equipment, including open-kettle, continuous-flow ribbon, and Electropure pasteurizers. The efficiency of each treatment was determined by plate counts before and after pasteurization. The cold-pressed juices from the several varieties of grapes and the one variety of apple were brought to approximately the same temperature, 10°C.(50°F.), before pasteurization.

In order to determine the effects of different flash-pasteurizing temperatures on the microorganisms in grape juice, six gallons of Delaware juice were run through a continuous-flow ribbon pasteurizer consisting of a steam-jacketed coil and a water-cooled coil. This equipment was first described by Von Loesecke, Mottern, and Pulley (1934) and later by Heid and Scott (1937). The juice was heated, beginning at a temperature of 73.9°C.(165°F.), and the heat was decreased by steps of 5°F. to 54.4°C.(130°F.), then similarly increased to 76.7°C.(170°F.) by adjusting the steam-inlet valve. Samples taken at the outlet of the heating coil at the time of reaching each temperature were plated, using a yeast-extract-peptone-glucose-agar medium. Plates were incubated four days at 32°C.(89.6°F.), and the colonies produced by surviving microorganisms were counted.

Five-gallon lots of Delaware, Elvira, Catawba, Concord, and Baldwin juices were pasteurized by heating to the desired temperature in a 20-gallon, aluminum, steam-jacketed, open kettle, with continuous stirring throughout the heating period. The time required to reach the desired temperature was recorded in each case. Temperatures were obtained with an ordinary mercury thermometer. Samples of the heated juices taken immediately after reaching the pasteurizing temperature were plated as previously described and the number of microorganisms surviving each temperature determined. After sampling, the pasteurized juices of Elvira, Delaware, and Catawba were run into five-gallon carboys, corked, and carefully cooled with water. Duplicate lots of the pasteurized juices of Concord and Baldwin were similarly run into carboys, some of which were immediately cooled with water, while others were allowed to air-cool gradually in order to determine the effects of prolonged heating.

Five-gallon lots of each juice were run through a continuous-flow ribbon pasteurizer and through a small continuous-flow Electropure pasteurizer of the type used in pasteurization of milk. In each case the containers of unpasteurized juice were placed approximately six feet above the apparatus and the contents allowed to flow by gravity

through the pasteurizers. Temperature readings of the juices flowing out of the steam-heated coil of the ribbon pasteurizer were taken by means of a thermocouple connected with an electro-potentiometric thermometer. The different temperatures were obtained by adjusting the inlet valve on the steam jacket. Temperature readings in the Electropure pasteurizer were taken by means of a mercury thermometer with the stem inserted in the outflowing juice. The different temperatures were obtained by adjusting the rate of flow of the juice. In each case samples for microbial counts were taken at the outlet tube after at least two gallons of heated juice had been collected. The pasteurized juices of Elvira, Delaware, and Catawba grapes were run into carboys and corked, those from the ribbon pasteurizer first being cooled by passing through the water-jacketed coil, and those from the Electropure pasteurizer being cooled immediately with water. Duplicate lots of the pasteurized juices of Concord grapes and Baldwin apples were similarly treated, with the exception that some lots were run directly into carboys, corked, and allowed to air-cool gradually. In addition to the samples for microbial counts taken at the time of heating, the pasteurized juices of Catawba and Concord grapes and Baldwin apples were again sampled after 24 hours in order to determine any increase in the number of microorganisms which had survived the various temperatures. The pasteurized Concord juices were also sampled for surviving microorganisms 48 hours after heating.

All lots of pasteurized juices, as well as unpasteurized controls, were inoculated with a pure-culture wine yeast and allowed to ferment at 21.1°C. (70°F.) for approximately six weeks. Unpasteurized controls of each juice were allowed to ferment spontaneously by the natural microorganisms present for comparative purposes. Previous to fermentation the grape juices were adjusted to approximately the same degree of sweetness (22° Brix) by addition of cane sugar. No sugar was added to the apple juices. After fermentation the wines were removed to the cellar at a temperature of 12.8 to 15.5°C. (55 to 60°F.) and left approximately five months. These wines were then filtered and samples analyzed for alcohol, total sugar, total acid as tartaric, volatile acid, and total protein; the specific gravity and hydrogen-ion concentration were also determined. Samples of the original unheated juices of each variety were analyzed for total sugar, total acid as tartaric, and total protein; the specific gravity, Brix reading, and hydrogen-ion concentration were obtained. The finished wines were submitted to organoleptic tests to detect any cooked or "off" flavors.

DISCUSSION OF RESULTS

Since these studies were undertaken to determine the temperatures necessary to kill all microorganisms in fruit juices for storage purposes, and also the temperatures necessary to reduce the number of microorganisms for pure-culture fermentations, heating a juice in a continuous-flow pasteurizer over a wide range of temperatures would yield results on the killing of microorganisms at each temperature used. Pasteurization of Delaware juice in a ribbon pasteurizer at 73.9°C. (165°F.) was sufficient to kill all microorganisms (Table 1),

TABLE 1
Effect of Temperature in a Ribbon Pasteurizer on Delaware Grape Juice

Temperature	Plate count ¹
°F.	per c.c.
165	0
160	1 (mold)
155	31 (9 molds)
150	60 (30 molds)
145	131
140	553
135	2,800
130	12,000
	580,000 (original count)
135	1,000
140	436
145	266
150	42
155	11 (6 molds)
160	1 (mold)
165	0
170	0

¹ Plate counts are from samples of a continuous flow of juice, temperature being varied by adjustment of steam-inlet valve. Approximate time of exposure to pasteurization temperature was 15 seconds.

but as the temperature was lowered by steps of 5°F. to 54.4°C. (130°F.), the number of microorganisms became increasingly larger. As the temperature was again increased by steps of 5°F. to 76.7°C. (170°F.), the number of microorganisms decreased correspondingly until finally all were killed at 73.9°C. It will be noted that in both cases heating to 73.9°C. killed all microorganisms, so the juices thus treated should remain sterile for storage purposes. Pasteurization at 60°C. (140°F.) was sufficient to kill a majority of the undesirable microorganisms, so that a pure-culture yeast inoculum would predominate in the fermentation. Pasteurization at temperatures as low as 54.4°C. and 57.2°C. caused a marked reduction in the number of microorganisms present, the efficiency of the killing effect amounting to 97 and 99 per cent, respectively.

Microorganisms surviving heating between 65.5°C.(150°F.) and 71.1°C.(160°F.) were identified as mold and spore-forming types; and although their presence is undesirable in fruit juices, it is unlikely they were capable of causing spoilage or fermentation in juices properly handled before storage.

Studies on the pasteurization of all juices heated in the open-kettle, ribbon, and Electropure pasteurizers at different temperatures gave similar results. Pasteurization of Elvira, Delaware, and Catawba juices at 60°C.(140°F.) in the three types of equipment was sufficient to cause a marked reduction in the number of microorganisms (Table 2). At this temperature the most complete killing of microorganisms was obtained with the open-kettle method, owing to the length of time necessary to heat a five-gallon lot of juice to the desired pasteurization temperature. In the continuous-flow ribbon and Electropure pasteurizers the juices were exposed to heating only for the few seconds necessary for them to pass through the apparatus. Plate counts from samples of Catawba juice taken 24 hours after heating in the three pasteurizers at 60°C.(140°F.) showed that the organisms did not increase in number in the juices that had been heated in the open kettle. A considerable increase in the number of microorganisms occurred in those juices heated in the ribbon and Electropure pasteurizers. There was no marked difference between plate counts from samples of Elvira, Delaware, and Catawba juices heated in the ribbon pasteurizer at 65.5°C.(150°F.) when compared with those heated in the three types of pasteurizers at 60°C. (Table 2). All microorganisms were killed when these juices were heated to 73.9°C.(165°F.) in the open kettle. A few organisms survived this temperature in the ribbon and Electropure pasteurizers. The lots of Catawba juices remained practically sterile 24 hours after heating as indicated by plate counts from samples of each. This was especially true in the case of the juice heated in the open kettle at 73.9°C.

In order to compare the effects of prolonged heating caused by not quickly cooling the juice on the number of microorganisms surviving pasteurization, duplicate lots of Concord juice were heated to 60°C.(140°F.) and 73.9°C.(165°F.) in the three pasteurizers. Effective pasteurization was obtained in the open-kettle and Electropure pasteurizers at 60°C.(140°F.) but not in the ribbon pasteurizer (Table 3). When all pasteurized juices were sampled 24 hours later, however, the effects of the slow cooling were clearly indicated. Those juices not cooled immediately after pasteurization were practically sterile, while those which were cooled showed a marked increase in the number of microorganisms. This was also demonstrated when lots of Concord juice were heated to 73.9°C.(165°F.). Samples taken

TABLE 2
Plate Counts Obtained in Pasteurization of Juices of Elvira, Delaware, and Catawba Grapes

Treatment	Time of exposure to pasteurization temperature			Plate count after various treatments			Plate count ¹ after 24 hrs.
	Elvira	Delaware	Catawba	Elvira per c.c.	Delaware per c.c.	Catawba per c.c.	
Unpasteurized control.....	9,000,000	580,000	3,560,000	per c.c.
Ribbon pasteurizer, 140° F.	16 sec.	9 sec.	587	1,400	5,000
Electropure, 140° F.	21 sec.	16 sec.	80	9	188	25,000
Open kettle, 140° F.	13 min.	12 min.	7.5 min.	1	39	49	42
Ribbon pasteurizer, 150° F.	17 sec.	9 sec.	80	56	65	105
Ribbon pasteurizer, 165° F.	12 sec.	8 sec.	28	22	2	7
Electropure, 165° F.	21 sec.	23 sec.	2	1	0	3
Open kettle, 165° F.	12 min.	11 min.	10.5 min.	0	0	0	0

¹ Plate counts were obtained from samples of juice which had been run into clean but not sterile glass carboys.

TABLE 3
Plate Counts Obtained in Pasteurization of Concord Grape Juice

Treatment	Time of exposure to pasteurization temperature	Plate count after various treatments	Plate count ¹ after 24 hrs.	Plate count ¹ after 48 hrs.
		per c.c.	per c.c.	per c.c.
Unpasteurized control.....	600,000
Ribbon pasteurizer, 140° F. (cooled).....	9 sec.	20,000	10,000	33,000
Ribbon pasteurizer, 140° F. (uncooled).....	13 sec.	4,400	6	280
Electropure, 140° F. (cooled).....	15 sec.	560	12,000
Electropure, 140° F. (uncooled).....	19 sec.	19	6
Open kettle, 140° F. (cooled).....	9 min.	13	286
Open kettle, 140° F. (uncooled).....	5 min.	3	0	42
Ribbon pasteurizer, 165° F. (cooled).....	9 sec.	1	104	2,600
Ribbon pasteurizer, 165° F. (uncooled).....	7 sec.	1	2	1
Electropure, 165° F. (cooled).....	22 sec.	7	7,000
Open kettle, 165° F. (cooled).....	10 min.	0	18,000	42,500
Open kettle, 165° F. (uncooled).....	9.5 min.	0	2	5

¹ Plate counts were obtained from samples of juice which had been run into clean but not sterile glass carboys.

after heating contained but few organisms, while those taken 24 and 48 hours later showed that those lots not cooled remained nearly sterile. Those lots which were cooled showed increases in the number of surviving microorganisms.

Pasteurization of duplicate lots of Baldwin apple juice at 65.5°C. (150°F.) resulted in a marked reduction in the number of microorganisms (Table 4). After 24 hours plate counts showed that the one lot not cooled after heating remained practically sterile, while those lots which were cooled showed increases in the number of surviving microorganisms. Heating to a temperature of 76.7°C. (170°F.) in the three pasteurizers practically sterilized the lots of juice, but

TABLE 4
Plate Counts Obtained in Pasteurization of Baldwin Apple Juice

Treatment	Time of exposure to pasteurization temperature	Plate counts after various treatments	Plate counts ¹ after 24 hrs.
		<i>per c.c.</i>	<i>per c.c.</i>
Unpasteurized control.....	377,000	600,000
Ribbon pasteurizer, 150°F. (cooled).....	28 sec.	12,000	11,000
Ribbon pasteurizer, 150°F. (uncooled)....	18 sec.	4	3
Electropure, 150°F. (cooled).....	27 sec.	14	10,000
Open kettle, 150°F. (cooled).....	15 min.	500
Ribbon pasteurizer, 170°F. (cooled).....	18 sec.	180	2,000
Ribbon pasteurizer, 170°F. (uncooled)....	15 sec.	1	3
Electropure, 170°F. (cooled).....	27 sec.	0	9,600
Open kettle, 170°F. (cooled).....	12 min.	1	1,300

¹ Plate counts were obtained from samples of juice which had been run into clean but not sterile glass carboys.

again it was only the one lot not cooled after heating that remained nearly sterile after 24 hours.

Results obtained from plate counts of samples of juices taken 24 hours after pasteurization (Tables 2, 3, and 4) suggest a point of possible commercial value. It is a common practice in some wineries to allow freshly pressed grape juice to stand overnight in tanks in order to allow suspended material to settle out, thus leaving a fairly clear juice which is more easily clarified after fermentation. It is possible in this comparatively short time for fermentation to begin which would interfere with sedimentation owing to the evolution of carbon dioxide gas. It has been found necessary in this respect to add sufficient sulfur dioxide to prevent fermentation. More efficient results possibly could be obtained by pasteurizing the freshly pressed juice in a suitable continuous-flow pasteurizer without cooling. The heated juice would then be run into a storage tank and allowed to

TABLE 5
Chemical Analyses of Juices From Different Grape and Apple Varieties, 1935

Variety	Specific gravity	Total sugar, dextrose	Brix reading at 17.5°C. (63.5°F.)	Total acid, tartaric	Hydrogen-ion concentration	Protein
Elvira.....	1.063	gm./100 c.c. 12.6	deg. 15.5	gm./100 c.c. 1.48	2.95	gm./100 c.c. .334
Delaware.....	1.078	15.3	19.0	1.23	3.06	.295
Concord (Fredonia, N. Y.).....	1.072	13.3	17.5	0.94	3.03	.341
Concord (Hammondsport, N. Y.).....	1.059	10.7	14.5	1.36	2.98	.424
Catawba.....	1.076	14.0	18.5	1.72	2.85	.421
Baldwin (apple).....	1.051	9.8	12.6	0.51 (malic)	3.30	.091

settle and cool overnight, after which the clarified juice would be drawn off and inoculated with a starter of wine yeast.

No direct relationship could be noted between the decrease in number of living microorganisms during pasteurization and the chemical analyses of the original untreated grape juices (Table 5). The Baldwin apple juice which had a lower acidity and higher pH than the grape juice did require higher temperatures for pasteurization.

A careful examination of the chemical analyses of the finished wines made from unpasteurized, inoculated and uninoculated control juices when compared with those made from juices pasteurized at different temperatures and then inoculated show only slight differences (Tables 6 and 7). Pasteurization of the grape and apple juices at temperatures ranging from 60 to 76.7°C. (140 to 170°F.) in the three types of pasteurizers had no detrimental effects on the fermentability and chemical composition of the finished wines. The fermentations of the heated juices were as complete as those of the unheated control juices. Excessive heating of fruit juices may cause a coagulation of proteins or a change of other chemical constituents which may bring about a faulty or incomplete fermentation.

Organoleptic tests conducted on the finished wines by commercial winery men failed to detect any great differences between flavor and aroma of the various samples. No cooked or "off" flavors were noted. It was the consensus of opinion that the samples of wine made from pasteurized juices seemed to be more mellowed and aged than those made from unpasteurized juices. All wine samples were judged as being of good, sound quality.

SUMMARY

Studies were made on the pasteurization of freshly pressed juices of Elvira, Delaware, Catawba, and Concord grapes and of Baldwin apples in several types of pasteurizing equipment including the open-kettle, ribbon, and Electropure pasteurizers. Various temperatures were used ranging from 54.4°C. (130°F.) to 76.7°C. (170°F.), and the killing of microorganisms was determined by platings of samples taken before and after each heat treatment.

Pasteurization of a single lot of Delaware grape juice was carried out in a continuous-flow ribbon pasteurizer beginning at a temperature of 73.9°C. (165°F.), decreased to 54.4°C. (130°F.) by 5°-F. changes, and then increased to 76.7°C. (170°F.) in the same manner. All microorganisms were killed at 73.9°C., but as the temperature was lowered the number of surviving microorganisms became increasingly larger. As the temperature was increased, the number of micro-

TABLE 6

Chemical Analyses of Elvira, Delaware, Catawba, and Concord Grape Wines Made From Pasteurized Juices

Treatment	Specific gravity				Alcohol (pct. by vol.)				Total sugar, dextrose (gm./100c.c.)			
	Elvira	Delaware	Catawba		Elvira	Delaware	Catawba		Elvira	Delaware	Catawba	
Unpasteurized, uninoculated ¹991	.990	.992		11.84	12.18	13.26		1.15	.572	0.86	
Unpasteurized, inoculated.....	.992	.989	.992		11.76	12.40	13.10		1.09	.469	0.99	
Ribbon pasteurizer, 140° F.....990	.992		12.04	12.96	433	0.76	
Electropure, 140° F.....	.989	.991	.993		11.98	12.02	13.02		0.345	.608	1.25	
Open kettle, 140° F.....	.990	.988	.994		12.14	12.35	13.30		0.375	.605	1.19	
Ribbon pasteurizer, 150° F.....	.989	.990	.993		12.04	12.34	12.82		0.342	.324	1.07	
Ribbon pasteurizer, 165° F.....	.989	.989	.991		11.90	12.02	13.10		0.378	.426	0.81	
Electropure, 165° F.....	.989	.989	.993		12.12	12.28	12.82		0.343	.403	1.21	
Open kettle, 165° F.....	.989	.989	.994		12.28	12.40	13.16		0.329	.345	1.34	
	Concord				Concord				Concord			
Unpasteurized, uninoculated ¹996				11.70				1.210		
Unpasteurized, inoculated.....		.993				12.56				1.260		
Ribbon pasteurizer, 140° F. (cooled).....		.993				12.51				1.380		
Ribbon pasteurizer, 140° F. (uncooled)....		.992				12.46				1.092		
Electropure, 140° F. (cooled) ²993				12.79				1.098		
Electropure, 140° F. (uncooled) ²991				12.40				0.920		
Open kettle, 140° F. (cooled).....		.992				12.99				0.859		
Open kettle, 140° F. (uncooled).....		.990				12.99				0.804		
Ribbon pasteurizer, 165° F. (cooled).....		.992				12.76				1.916		
Ribbon pasteurizer, 165° F. (uncooled)....		.990				12.04				1.825		
Electropure, 165° F. (cooled) ²993				12.68				1.047		
Open kettle, 165° F. (cooled).....		.991				12.85				0.801		
Open kettle, 165° F. (uncooled).....		.990				13.41				0.753		

¹ These lots were allowed to ferment spontaneously by natural microorganisms, whereas others were fermented by addition of pure-culture wine yeast. ² Hammondsport stock.

TABLE 6 (Concluded)
Chemical Analyses of Elvira, Delaware, Catawba, and Concord Grape Wines Made From Pasteurised Juices

Treatment	Total acid, tartaric (gm./100 c.c.)				Volatile acid, acetic (gm./100 c.c.)				Hydrogen-ion concentration				Protein (gm./100 c.c.)			
	Elvira	Delaware	Catawba	Elvira	Delaware	Catawba	Elvira	Delaware	Catawba	Elvira	Delaware	Catawba	Elvira	Delaware	Catawba	Elvira
Unpasteurized, uninoculated ¹77	.70	1.03	.069	.039	.051	2.96	3.08	2.73	.171	.225	.184	.171	.225	.184	.171
Unpasteurized, inoculated.....	.76	.71	1.01	.048	.058	.066	2.93	3.08	2.73	.149	.204	.166	.149	.204	.166	.149
Ribbon pasteurizer, 140° F.....74	1.11060	.059	3.03	2.73147	.188147	.188
Electropure, 140° F.....	.78	.73	0.94	.051	.060	.066	2.93	2.85	2.76	.136	.186	.190	.136	.186	.190	.136
Open kettle, 140° F.....	.79	.76	1.35	.063	.066	.057	2.90	3.00	2.64	.166	.186	.230	.166	.186	.230	.166
Ribbon pasteurizer, 150° F.....	.80	.71	1.00	.054	.052	.072	2.86	3.03	2.77	.127	.190	.197	.127	.190	.197	.127
Ribbon pasteurizer, 165° F.....	.79	.81	0.97	.063	.054	.048	2.91	3.05	2.79	.136	.173	.192	.136	.173	.192	.136
Electropure, 165° F.....	.76	.73	1.05	.054	.058	.075	2.91	3.05	2.75	.157	.220	.190	.157	.220	.190	.157
Open kettle, 165° F.....	.79	.76	1.09	.048	.064	.059	2.87	3.03	2.75	.171	.190	.190	.171	.190	.190	.171
Concord																
Unpasteurized, uninoculated ¹850			.111			2.94				.161					
Unpasteurized, inoculated.....	.705			.093			3.11				.143					
Ribbon pasteurizer, 140° F. (cooled).....	.720			.057			2.99				.162					
Ribbon pasteurizer, 140° F. (uncooled)....	.720			.085			3.01				.183					
Electropure, 140° F. (cooled) ²814			.058			2.90				.228					
Electropure, 140° F. (uncooled) ²820			.066			2.92				.199					
Open kettle, 140° F. (cooled).....	.740			.078			2.99				.143					
Open kettle, 140° F. (uncooled).....	.727			.090			2.96				.135					
Ribbon pasteurizer, 165° F. (cooled).....	.750			.078			3.14				.157					
Ribbon pasteurizer, 165° F. (uncooled)....	.730			.093			2.99				.167					
Electropure, 165° F. (cooled) ²840			.063			2.89				.226					
Open kettle, 165° F. (cooled).....	.730			.093			2.99				.178					
Open kettle, 165° F. (uncooled).....	.727			.051			2.99				.170					

¹ These lots were allowed to ferment spontaneously by natural microorganisms, whereas others were fermented by addition of pure-culture wine yeast. ² Hand-sports stock.

TABLE 7

Chemical Analysis of Baldwin Apple Cider Made From Pasteurized Juice

Treatment	Specific gravity	Alcohol pct. by vol.	Total sugar, dextrose gm./100 c.c.	Total acid, malic gm./100 c.c.	Volatile acid, acetic gm./100 c.c.	Hydrogen-ion concentration	Protein gm./100 c.c.
Unpasteurized, uninoculated ¹996	6.62	.23	.48	.135	3.55	.022
Unpasteurized, inoculated995	6.79	.09	.48	.135	3.55	.035
Ribbon pasteurizer, 150° F. (cooled)999	6.27	.18	.62	.207	3.44	.035
Ribbon pasteurizer, 150° F. (uncooled) ..	.999	6.00	.43	.58	.176	3.50	.035
Electropure, 150° F. (cooled)999	6.37	.67	.54	.156	3.48	.026
Open kettle, 150° F. (cooled)996	6.69	.20	.42	.105	3.60	.031
Ribbon pasteurizer, 170° F. (cooled)998	6.20	.45	.58	.099	3.49	.035
Ribbon pasteurizer, 170° F. (uncooled) ..	.998	6.49	.36	.56	.078	3.53	.017
Electropure, 170° F. (cooled)999	6.23	.35	.58	.177	3.26	.022
Open kettle, 170° F. (cooled)999	6.27	.69	.38	.090	3.53	.035

¹ These lots were allowed to ferment spontaneously by natural microorganisms, whereas others were fermented by addition of pure-culture wine yeast.

organisms decreased correspondingly until finally all were killed at 73.9°C.

In general, pasteurization at a temperature of 60°C.(140°F.) in the three types of pasteurizers was sufficient to cause a marked reduction in the number of microorganisms in the Elvira, Delaware, Catawba, and Concord juices, so that a pure-culture inoculum of wine yeast when added would predominate and control the fermentation. All microorganisms were killed when these juices were heated to 73.9°C.(165°F.) in the open kettle. A few organisms survived this temperature after heating in the ribbon and Electropure pasteurizers, but in most cases they were not types capable of causing spoilage or fermentation if the heated juices were properly handled. Heating Baldwin apple juice to temperatures of 65.5°C.(150°F.) and 76.7°C.(170°F.), respectively, gave results similar to those obtained with the grape juices.

Pasteurized juices of Concord grapes and Baldwin apples allowed to cool gradually after heating showed, after 24 hours, a greater killing of microorganisms than did those lots cooled immediately. This was due to the effect of prolonged heating during the slow cooling process.

Results from plate counts of samples of juices taken 24 hours after heating suggest the possibility of using low-temperature pasteurization for fruit juices which are to be held in storage for short periods to allow sedimentation of suspended material.

There were only slight differences in the chemical compositions of wines made from the various pasteurized juices, and organoleptic tests of these wines failed to give any indication of cooked or "off" flavors.

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PECTIC ENZYMES. II. PECTIC ENZYMES OF TOMATOES ¹

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The pectic materials of tomatoes contribute greatly to the consistency, thickness, or "body" of various tomato products. The canner prefers tomatoes in the fully ripened stage of maturity when their pectin content is highest, and he takes precautions to conserve the natural pectins during the canning process.

Appleman and Conrad (1927) found a direct correlation between amount of pectic materials and quality of tomatoes. This was confirmed by Saywell and Cruess (1932) who also found considerable variation between varieties. LeCrone and Haber (1933) studied the changes in stored tomatoes and found that a decrease in the pectic constituents coincided with softening and subsequent deterioration. Rooker (1930) investigated the effect of pectin on the quality of catsup and advocates the addition of pectin during manufacturing.

The presence of enzymes in tomatoes leads to the use of the "hot break" method of processing, in which the fruit is heated to inactivate the natural pectic enzymes before cycloning. Wildman (1930) showed that the advantage of the "hot break" method lies not in the extraction of pectinous materials from the seed, as postulated by Smith (1931) and others, but in the preservation of the natural pectins. Wildman concluded that in crushed, unheated tomatoes about 70 per cent of the pectin is lost in 10 minutes. With the exception of this data no information is available concerning the presence and activity of pectic enzymes in tomatoes. In the present paper experimental results obtained on these two questions are presented.

(Three pectic enzymes may influence the quality of tomato products: protopectinase which is presumed to act upon the insoluble protopectin of tomatoes converting it into soluble pectin and other products; pectin-methoxylase (pectase) which catalyzes the demethoxylation of the soluble pectin; the pectin-polygalacturonase which is responsible for the decomposition of the polygalacturonic acid nucleus of pectin, as described by Kertesz (1936). The present report deals only with pectin-methoxylase and pectin-polygalacturonase of tomatoes.)

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PECTIN-METHOXYLASE

Pectin-methoxylase (pectase) catalyzes the cleavage of methoxyl groups from pectin, resulting in the formation of methanol and pectic acid. The juice expressed from the tomatoes was used for the determination of the enzyme. The fruits were cut, wrapped in a cloth, and the juice squeezed out manually. Only a small quantity of skin and seeds remained on the cloth. Experiments with filtered or centrifuged tomato juice indicated that a considerable portion of the en-

TABLE 1
Pectin-Methoxylase Activity in Tomatoes

Variety	Date	Pectin-methoxylase units per gram		
		Green	Almost ripe	Ripe
(Shipped from Florida)	May 21	58
	May 25	97
	May 25	105
Forcing Wonder (grown in glass house)	May 21	29
	May 25	120
	June 1	44	86	114
	June 3	101
	June 14	49	77
	June 16	143
	June 22	149
	June 29	35	83	135
John Baer	July 29	187
	Aug. 2	52	163	188
Nystate	Aug. 2	197
	Aug. 4	82
Kobourg	Aug. 4	184
King Humbert	Aug. 4	114	142

zyme is in the suspended cellular tissues and therefore the juice was used as expressed. Holding the juice for several days under toluol at 0°C.(32°F.) had practically no effect on the pectin-methoxylase activity. For the determination of the enzyme action the method described by Kertesz (1937) was used. The activity of the enzyme is expressed in pectin-methoxylase units per gram of dry material in the enzyme solution used. One gram of material containing one pectin-methoxylase unit splits off one milligram of methoxyl under the above conditions.

The results obtained on six varieties of tomatoes are presented (Table 1). The Forcing Wonder tomatoes were grown in a glass house; the other varieties were grown in the field on stakes.

(Tomatoes have not previously been considered a good source of pectin-methoxylase. Determinations on material usually regarded as rich in pectin-methoxylase, such as the sap pressed from alfalfa and green tobacco leaves, gave results not exceeding 50 pectin-methoxylase units per gram. The enzyme activity of the juice from green tomatoes is of this order. Partially ripe tomatoes gave values in the range of 80-120 pectin-methoxylase units, while the ripe fruit contained (with the exception of the Florida tomatoes) 115-195 pectin-methoxylase units per gram.)

From these figures the time required for the complete demethoxylation of the natural pectin in tomatoes can be calculated. (The average pectin content of ripe tomatoes is about .15 per cent and the maximum methoxyl content of this pectin in the neighborhood of 10 per cent. The pH of the ripe tomatoes is approximately 4.2. At this pH the velocity of the demethoxylation was found by Kertesz (1937) to be only 15 per cent of that occurring at pH 6.2. In the cold-pressed juice of ripe tomatoes having an enzyme activity of 180 pectin-methoxylase units the demethoxylation should be complete at room temperatures in less than four minutes. It is obvious that no heat inactivation of the enzyme in the cold-pressed juice is possible without considerable demethoxylation. The practical significance of this reaction will be discussed later in this article.

PECTIN-POLYGALACTURONASE

Pectin-polygalacturonase is the most important member of the pectinase complex which contains enzymes decomposing pectins into their simple constituents, as noted by Kertesz (1936). The pectin-polygalacturonase splits the chain-like polygalacturonic acid structure of pectins. The method usually applied for the determination of pectin-polygalacturonase activity depends on the estimation of the terminal aldehyde groups liberated upon decomposition of the polygalacturonic acid chain. One gram of material containing one pectin-polygalacturonase unit produces one milligram galacturonic acid in 30 minutes at pH 3.5 at 30°C. (86°F.).

(The highest value obtained in tomato juice by this method was 18 pectin-polygalacturonase units. Because of the low activity large proportions of tomato juice had to be used in the determination. Tomato juice contains about three per cent sugar which makes the determination of an additional small reducing power uncertain. Good sources of pectin-polygalacturonase as malt extract and commercial pectinases contain 200 to 700 pectin-polygalacturonase units.)

To obtain additional information on the presence or absence of pectin-polygalacturonase in tomatoes a number of reaction mixtures

containing 30 cubic centimeters of an approximate one-per cent citrus-pectin solution and 10 cubic centimeters of tomato juice were set up. The decomposition of the pectin was followed by precipitating the mixture with 800 cubic centimeters of 95 per cent alcohol. Determinations were made at intervals up to 40 days. Toluol was used in the mixtures to prevent bacterial contamination. The pH of the reaction mixtures was between 3.4 and 3.7 which is the optimum region for the enzyme.

The decrease in the alcohol precipitate caused by the juice of green tomatoes was always less than six per cent. A demethoxylation of 10 per cent might be ascribed to the pectin-methoxylase present, thus pectin-polygalacturonase was apparently absent from green tomatoes. From eight reaction mixtures where partly ripe and ripe tomatoes were used, four showed a decrease of less than four per cent during holding for a number of days. In the other four samples a decrease could be observed, the highest being a decrease of 40 per cent in 40 days. For these tests, tomatoes of three varieties (Forcing, Wonder, Nystate, and John Baer) were used. The presence of pectin-polygalacturonase did not appear to be a varietal characteristic. The highest activity found by this method calculated and expressed in pectin-polygalacturonase units is less than five pectin-polygalacturonase units and thus negligible when compared with the very high pectin-methoxylase activity of the same tomatoes.

{ The observation that pectin-polygalacturonase is absent from green tomatoes and is not always detectable in ripe ones raises an interesting question regarding the identity of protopectinase and pectinase. The hypothesis that the two enzymes are identical has been often advocated but no experimental evidence was ever presented to prove or definitely disprove this supposition. Appleman and Conrad (1927) showed that the transformation of the protopectin into soluble pectin takes place in green tomatoes as well as in partially and fully ripe ones. Thus the observed absence of pectinase (or more correctly that of the pectin-polygalacturonase) is indirect evidence that the two enzymes cannot be identical.

CAUSE OF CHANGES IN VISCOSITY OF COLD-PRESSED TOMATO JUICE

It appears from these results that the damage done to the pectins of tomatoes during preparation consists entirely of a demethoxylation and not of an actual decomposition of the pectin nucleus by the enzymes of the pectinase complex. A decrease in the viscosity of pectin solutions has been presumed by Willaman (1927) and others to be caused by a decomposition of the polygalacturonic acid nucleus, although Von Fellenberg (1918) made the statement 19 years ago

that the action of the pectin methoxylase alone may lower the viscosity of pectin solutions.

To prove the validity of this statement in connection with the present work one cubic centimeter of heated tomato juice was added to 25 cubic centimeters of an approximate one-per cent pectin solution and the pH adjusted to 6.2 with 0.1N NaOH, the mixture made up to 50 cubic centimeters, and the viscosity measured in an Ostwald pipette at 30°C. (86°F.). To another 25 cubic centimeters of the same pectin solution, one cubic centimeter of the unheated tomato juice was added and the pH maintained at nearly 6.2 by stepwise additions of alkali until complete demethoxylation had occurred. Then the mixture was made up to 50 cubic centimeters and the viscosity measured in the other sample. The relative viscosity as compared with water at 30°C. decreased during demethoxylation from 2.90 to 2.30.

The pectin-methoxylase caused a loss of about one-fifth of the original viscosity. The decrease is also great in the tomato juice itself, without the addition of pectin. The following results were obtained in tomato juice which was filtered immediately after pressing. As mentioned earlier in this paper the filtration removes a considerable portion of the suspended material and the enzyme activity in the filtered juice is much lower. The results of viscosity determinations are presented (Table 2).

TABLE 2
*Changes in Relative Viscosity of Filtered Cold-Pressed
Tomato Juice*

Time from pressing	Relative viscosity compared with water at 30°C. (86°F.)
min.	
0	3.00
14	2.15
29	1.82
47	1.64
69	1.63
159	1.62
360	1.62

It appears from the data (Table 2) that the viscosity of the filtered tomato juice was decreasing for about 40 minutes but after that was practically constant. That the decrease in the viscosity was caused by demethoxylation and not by the action of pectin-polygalacturonase was also indicated by the formation of calcium-pectate precipitates when calcium-chloride solution was added. If tomato-juice

samples standing as pressed were heated and filtered in five-minute intervals, the decrease of the viscosity took place during the first five minutes and there was but little change later. Naturally the observed decrease in the viscosity will be greatly influenced by the quantity of available calcium present because the calcium salt of pectic acid has a much higher viscosity in equal concentration than the free pectic acid.

These findings are in agreement with the general conclusions of Wildman (1930) regarding the effect of natural enzymes on the quality of cold-pressed tomato juice. Wildman found, however, that if cold-pressed tomato juice is held and filtered after 10 minutes' standing, there is a great loss in the pectin content of the juice, amounting to as much as 96 per cent in certain juices. The author believes that the pectic acid formed from the pectin was lost in Wildman's samples during the filtration. Pectic acid is rather insoluble in water and, especially if there were any opportunity for the formation of calcium pectate, the pectic acid would have remained on the filter paper with the cellular tissues. Although Wildman's results could not be duplicated in this laboratory, the filter always retained a portion of the pectic acid formed. In one experiment, for instance, the loss of pectic acid was in the neighborhood of 15 to 18 per cent after the tomato juice stood for 5, 10, and 15 minutes. But even after five days 66 per cent of the original pectin could be determined in the filtrate as pectic acid. Possibly different conditions of filtration are the cause of this discrepancy between Wildman's results and those given here.

SUMMARY AND CONCLUSIONS

1. Pectin-methoxylase (pectase) was found to be present in all tomato samples investigated. The activity found in green tomatoes is about 40-50 pectin-methoxylase units but increases to 80-120 pectin-methoxylase units as the fruit approaches maturity and reaches 180-190 pectin-methoxylase units in ripe field tomatoes.

2. Pectin-polygalacturonase (the most important enzyme of the pectinase complex) is practically absent from green tomatoes. In about half of the riper tomatoes investigated the enzyme could be found but with extremely low activity.

3. The changes in the viscosity (and quality) of cold-pressed tomato juice probably are not due to the decomposition of natural pectins by enzymes of the pectinase group but are caused entirely by the rapid enzymic demethoxylation of the pectin.

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VITAMIN VALUES OF GARDEN-TYPE PEAS PRESERVED BY FROZEN-PACK METHOD. I. ASCORBIC ACID (VITAMIN C)

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Fresh green peas are recognized as a good source of ascorbic acid, but there have been comparatively few studies on the influence of commercial handling and processing on the ascorbic acid content of this vegetable. Eddy, Kohman, and Carlsson (1926) and Fellers and Stepat (1936) have reported on the losses of ascorbic acid due to canning. Mack and Tressler (1936) have found variety and sieve size to be factors which influence the ascorbic acid content of fresh peas, and the cooking losses in fresh peas have been studied by Fenton, Tressler, and King (1936).

Fellers and Stepat (1936) have estimated that losses due to canning operations were 66 per cent of the original ascorbic acid and that defrosting for several hours resulted in a loss of 70 per cent of the amount present in the frozen peas.

Frozen-pack peas are of considerable commercial importance and are produced in large quantities in the Pacific Northwest. Differences in soil and climatic conditions are probably factors which influence ascorbic acid content; also the method of freezing which is used in the Northwest differs from those generally used in the eastern section of the country. It was therefore considered of importance to investigate the ascorbic acid content of garden-type peas preserved by the frozen-pack method. The investigation here reported covers the following phases of the problem: (a) the ascorbic acid content of peas of the same variety scalded at different temperatures and for varying periods of time before freezing; (b) distribution of ascorbic acid between the cotyledon and seed coat of the peas, and the effect of thawing the peas under different conditions of time and temperature; and (c) varietal differences in ascorbic acid content of frozen peas.

This investigation was undertaken at the suggestion of H. C. Diehl of the Frozen Pack Laboratory, U. S. Bureau of Chemistry and Soils, at Seattle, Washington, and was made possible through courtesy of the laboratory which provided the peas. Its personnel undertook the preparation and freezing of all the peas except those used in the

varietal studies. The latter were obtained from the experimental plots of the Bozeman Canning Company at Snohomish, Washington, and the material was processed under commercial conditions at the Monroe plant of that company, under observation by the laboratory personnel.

EXPERIMENTAL WORK

The peas for the first part of the investigation were all of the Telephone variety, and were grown on sandy loam soil. At maturity (July, 1936) they were harvested, vined, and quickly transported to the Frozen Pack Laboratory at Seattle; they were washed, scalded in boiling water (Table 2 gives time and temperature) and then cooled to about 12.8°C. (55°F.); filled into No. 2 cans, sealed without vacuum, and stored at -20.5°C. (-5°F.). The peas reached freezing point in about two hours and came to storage temperature in approximately 15 hours. The time elapsing between picking and freezing was never more than six hours. The peas were shipped to Pullman, Washington, two months later and stored at approximately -15°C. (5°F.).

Determination of Ascorbic Acid Content by Animal Assay: The method used was that of Sherman, La Mer, and Campbell (1922), with modifications as previously described by Todhunter (1936), using lemon juice of known ascorbic acid content as a standard of reference. In all, 42 animals were used, and negative-control animals receiving basal diet only survived 27 to 34 days with an average scurvy score of 16. Positive controls received in addition to the basal diet either 1 ml. or 1.5 ml. of fresh lemon juice daily six days a week; the lemon juice was fed from a glass syringe graduated in tenths of a milliliter. Weighed supplements of peas which had been thawed in a covered container in a water bath at 25 to 32°C. (77 to 89.6°F.), were fed daily six days a week, and were consumed at once by the animals.

Samples of the same peas were taken and the reducing value determined as described below, using 2,6 dichlorophenolindophenol. Peas of lot No. 1723 were fed during the fall, at levels of two, four, and six grams, and peas of lot No. 1734 were fed during the winter at levels of four and five grams. The results are summarized (Table 1).

For the first series using peas, lot No. 1723, the protective level as actually found by animal experiment was between four and six grams and as calculated from the titration values was 5.1 grams; for the second sample of peas, lot No. 1734, the protective level by animal assay was found to be between four and five grams and from titration values was calculated to be 4.2 grams. Since there was such good agreement by the two methods it was considered justifiable to make all

further determinations of ascorbic acid by titration with 2,6 dichlorophenolindophenol.

Determination of Ascorbic Acid by Titration With 2,6 Dichlorophenolindophenol: Tillman's titration method as modified by Bessey and King (1933) and Musulin and King (1936) was used. The dye solution was made by successive washing of .1 gram of 2,6 dichlorophenolindophenol (Eastman Kodak) using 200 ml. of warm water redistilled from glass, to which a small amount of phosphate buffer of pH 6.5 had been added. This solution was kept in a dark bottle and made fresh every three days. The dye was standardized

TABLE 1

Comparison of Ascorbic Acid Content of Frozen-Pack Peas (Telephone Variety) Determined by Animal-Feeding Methods and by Titration

Supplement fed	Number of animals	Animal-feeding method		Titration value for ascorbic acid	Calculated protective level ²
		Scurvy score and range	Protective level ¹		
Series I					
Lemon juice 1 ml.	3	9 (8-10)	0.524
Lemon juice 1.5 ml.	6	1 (0-1)	1.5 ml.	0.786	1.5 ml.
Peas, lot No. 1723, 2 gm.	3	12 (10-13)	0.308
Peas, lot No. 1723, 4 gm.	5	2 (1-3)	>4 gm.	0.616
Peas, lot No. 1723, 6 gm.	5	0 (0)	<6 gm.	0.924	5.1 gm.
Series II					
Lemon juice 1 ml.	4	2 (1-5)	0.503
Lemon juice 1.5 ml.	2	0 (1-0)	1.5 ml.	0.755	1.5 ml.
Peas, lot No. 1734, 4 gm.	6	1 (0-4)	>4 gm.	0.720
Peas, lot No. 1734, 5 gm.	4	0 (0-1)	<5 gm.	1.080	4.2 gm.

¹ Daily requirement by the guinea pig for protection from scurvy throughout the experimental period. ² Calculated weight of peas containing the same amount of ascorbic acid as in 1.5 ml. of lemon juice.

daily against lemon juice which had, in turn, been standardized against .01 N iodine solution. Since one ml. of .01 N iodine solution is equivalent to .88 mg. ascorbic acid the titer of the dye solution was obtained each day.

The cans of frozen peas were opened, and the solidly frozen contents were divided and put in four-ounce, screw-top jars and kept in the freezing unit of the refrigerator until required. The covered jars were thawed in a water bath at 25 to 32°C. (77 to 89.6°F.) (this usually required about one hour). Ten-gram samples of the thawed peas were ground in a glass mortar with 10 grams of sand (Baker's purified, washed, and ignited) plus 13 ml. of eight-per cent trichloroacetic acid and two ml. of 50-per cent metaphosphoric acid for one minute. The extract was centrifuged for one minute and decanted in-

TABLE 2

*Comparison of Ascorbic Acid Content of Frozen-Pack Telephone Peas, Size No. 6,
Soaked at Different Temperatures and for Varying Periods of Time*

Lot	Method of soaking	Number of cans used	Number of titrations (in duplicate)	Mean ascorbic acid and range of values <i>mg. per gm.</i>	Difference and P.E. of difference between means compared with No. 1724	R ¹
1724	Water, 1 min. 99°C.	2	6	.210 (.160-.259)
1725	Water, 2 min. 99°C.	3	8	.185 (.123-.230)	.025 ± .014	1.8
1726	Water, 3 min. 99°C.	3	8	.170 (.141-.198)	.040 ± .012	3.4
1723	Steam, 1 min. 99°C.	1	4	.188 (.178-.200)	.022 ± .011	2.0
1727	Water, 2 min. 88°C.	3	8	.212 (.163-.258)	.002 ± .013	0.2
1728	Water, 4 min. 88°C.	3	8	.232 (.197-.251)	.022 ± .011	2.0
1729	Water, 6 min. 88°C.	3	8	.225 (.196-.261)	.015 ± .011	1.3
1730	Water, 2 min. 71°C.	3	8	.081 (.047-.108)	.129 ± .012	11.2
1731	Water, 4 min. 71°C.	3	8	.072 (.036-.104)	.138 ± .012	11.0
1732	Water, 6 min. 71°C.	3	8	.091 (.037-.136)	.119 ± .014	8.6
1733	Held 4 hrs., 21°C., then as No. 1724	3	8	.161 (.127-.203)	.049 ± .012	4.2
1734	Held 8 hrs., 21°C., then as No. 1724	3	8	.206 (.141-.247)	.004 ± .013	0.3

¹ Statistical treatment is according to Chaddock (1925). R equal to or greater than 3, shows 21:1 chance that the difference is a true one. R equal to or greater than 4, shows 142:1 chance that the difference is a true one

to a volumetric flask. The mortar and residue were washed, and the washings were centrifuged and decanted into the first extract; the washing was again repeated, and each time 15 ml. of acid solution was used. The combined extractions were made up to 50 ml. Aliquots of 25 ml. were titrated with dye solution of known ascorbic acid titer, using a 10 ml. burette of narrow bore. The end-point was a pink color stable for 10 seconds. All procedures were timed with a stop watch and the titrations were completed in less than two minutes.

All ascorbic acid determinations reported in the remainder of this paper were determined by titration with 2,6 dichlorophenolindophenol as just described.

Ascorbic Acid Content of Telephone Peas Scalded at Different Temperatures and for Varying Periods of Time Before Freezing: In an attempt to secure adequate sampling, from three to eight cans of each lot of peas were taken and a total of 17 to 25 titrations made on each lot. A wide range in ascorbic acid values was obtained, indicating that representative sampling possibly had not been secured. The peas in the cans were not all of the same size; and Mack, Tressler, and King (1936) have found that the smaller peas showed a higher ascorbic acid content per unit of weight. Therefore, the influence of size in this particular variety of peas was determined.

The peas were sized after thawing: Size 4 peas were taken as those which passed through a sieve with holes $1\frac{1}{32}$ of an inch in diameter but not through holes of $1\frac{1}{32}$ of an inch in diameter; Size 5 peas passed through a $1\frac{3}{32}$ -inch sieve and Size 6 through a $1\frac{4}{32}$ -inch sieve; "larger than Size 6" was used to describe those peas which would not pass through a $1\frac{4}{32}$ -inch sieve. The number of peas of a given size varied in the different cans, but the average of three cans was as follows: 6.3 per cent of the weight of peas were smaller than Size 4; 11.4 per cent, Size 4; 19.7 per cent, Size 5; 30.8 per cent, Size 6; and greater than Size 6 made up 31.8 per cent of the total weight in the can which averaged 324 grams.

The ascorbic acid content of the peas, of Sizes 4, 5, 6, and larger than 6, was determined for four cans with the following average results: Size 4, .238 mg. per gram; Size 5, .224 mg. per gram; Size 6, .190 mg. per gram; and larger than Size 6, .164 mg. per gram. Larger peas of the same variety had definitely less ascorbic acid per gram than was contained in the smaller peas; therefore, all determinations of ascorbic acid content of the Telephone peas scalded for different periods of time and temperature were repeated using only Size 6 peas (Table 2).

Since it was not possible to make direct comparisons with fresh Telephone peas there was no measure of possible losses owing to the

process of scalding and freezing. It was possible, however, to compare the influence of different methods of treatment using lot No. 1724, scalded one minute in water at 99°C. (210.2°F.), as a standard for comparison. Data were treated statistically according to the method of Chaddock (1925). Lot No. 1723, scalded in steam for one minute, showed no significant difference in ascorbic acid content. Lot No. 1725, scalded two minutes in water at 99°C., was not significantly different from those scalded for one minute, but scalding three minutes at that temperature (lot No. 1726) was found to cause a significant reduction in ascorbic acid content. Kertesz, Dearborn, and Mack (1936) found the same amount of ascorbic acid retained in Thomas Laxton peas after one, two, and five minutes' scalding in steam and also that one-minute scalding was sufficient to inactivate ascorbic acid oxidase and catalase. Campbell and Diehl (1937) report that 30 to 60 seconds' scalding at 99°C. results in excellent quality in frozen-pack peas. There is no explanation at present for the decreased ascorbic acid content of the peas scalded for three minutes, since no increased destruction of ascorbic acid was found with a longer scalding period at other temperatures. The three lots of peas No. 1727, 1728, and 1729, scalded for 2, 4, and 6 minutes respectively at 88°C. (190.4°F.), showed no loss in ascorbic acid as compared with lot No. 1724; therefore it was concluded that this temperature and time was sufficient to inactivate any ascorbic acid oxidase.

Lots No. 1730, 1731, and 1732 scalded at 71°C. (159.8°F.) all showed a significant decrease in ascorbic acid, indicating that the oxidase possibly was not destroyed even in the six-minute period, or that there was destruction of ascorbic acid during the period of scalding. These peas were a yellowish-green color indicating either that some enzyme action had taken place or that the long period of scalding had caused a color change. Lot No. 1733 had been shelled and held for four hours at a room temperature of 21°C. (69.8°F.) before being scalded for one minute in water at 99°C. They showed a significant loss in ascorbic acid when compared with lot No. 1724. Peas of lot No. 1734 held for eight hours under the same conditions as lot No. 1733 showed no loss in ascorbic acid; these peas were also assayed by the animal-feeding method (Table 1), and ascorbic acid values determined by the two methods were the same. A possible explanation of this apparently contradictory information may be found in the work of Kohman and Sanborn (1937). These investigators found that freshly pressed green-pea juice held in the absence of air gradually increased its reducing value to 2,6 dichlorophenol-indophenol, and the effect was increased if the juice was first well aerated. Their data indicated that some substance, probably an enzyme capable of reducing dehydroascorbic acid, was involved

Ascorbic Acid Content of Cotyledon, Seed Coat, and Liquid Drained From Peas: Telephone variety peas, lot No. 1725, sieve size No. 6 (sized after thawing), were rapidly separated into cotyledons and seed coats and analyzed for ascorbic acid by dye titration. Peas were taken from four cans; three samples from each can were used and the average ascorbic acid content in the seed coat was found to be .207 mg. per gram with a range of .170 to .242 mg. per gram. In the cotyledon was found an average of .137 mg. ascorbic acid per gram, and the range was .116 to .161 mg. per gram. The seed coat was thus shown to have a higher ascorbic acid content per unit weight than the cotyledon. A similar difference in the peel and flesh of the apple has been reported by Todhunter (1936). It is possible that the higher vitamin C content observed in the smaller size peas may be due, at least in part, to the greater proportion of seed coat to cotyledon; this point is being further investigated.

Although the peas used in this investigation were dry packed, some water of cooling clung to them before freezing, and on thawing a small amount of liquid usually separated from the peas. Seven cans of lot No. 1728 were thawed at 32 to 35°C. (89.6 to 95°F.) for two hours; on opening the cans the peas were drained for one minute on a sieve (United States standard No. 8). The average content of the cans was 338 grams of peas, and 6.7 ml. of liquid. The liquid gave a reducing value, as measured by dye titration and calculated as ascorbic acid, of .414 mg. per ml. of liquid. Ascorbic acid is water-soluble; however, this was a high concentration to find in the liquid and further investigations are being made to determine whether or not this reducing value is entirely due to ascorbic acid.

Thawing Studies: Cans of peas were thawed as previously described using lot No. 1727. The peas which were titrated immediately after thawing showed an average ascorbic acid content of .170 mg. per gram of peas for eight samples (minimum .138, maximum .196); but when allowed to stand 30 minutes at room temperature, 24.7°C. (76°F.), the ascorbic acid content was .143 mg. per gram, a loss of 15.9 per cent. After standing one hour at room temperature there was only .124 mg. per gram, a loss of 27.1 per cent; peas which were thawed and then allowed to stand in the refrigerator at 4.5°C. (40°F.) for 24 hours had .128 mg. ascorbic acid per gram of peas, or a loss of 24.7 per cent.

These data show that frozen peas, if thawed and allowed to stand exposed to the air at room temperature for any length of time, rapidly lose ascorbic acid and that ordinary ice-box temperatures over a 24-hour period permit about the same loss as when the peas are held at room temperature for one hour.

Varietal Differences in Ascorbic Acid Content: The different varieties of peas for this study were grown on loam at Snohomish, Washington. They were harvested at maturity, vined, washed, scalded at 88 to 93°C. (190.4 to 199.4°F.) for one minute, cooled, and frozen in moving air at about -25°C. (-13°F.) on a freezing belt, reaching a temperature of -15°C. (5°F.) in 20 minutes, and immediately thereafter stored at -18°C. (-0.4°F.). Ascorbic acid was determined by titration with 2,6 dichlorophenolindophenol.

Different varieties of peas at the usual stage of maturity for commercial harvesting undoubtedly are not all of the same size, and in any one variety it has been shown that ascorbic acid content decreases with increasing size. For the varieties investigated here, how-

TABLE 3
*Ascorbic Acid Content of Several Varieties of Frozen Pack Peas—
Sieve Size No. 6*

Variety	Scalding temperature— 1 minute	Number of analyses	Ascorbic acid— mean value	Difference and P E of difference between means and Telephone variety	R ¹
	°C.		mg. per gm.		
Telephone.....	99	6	.210 ± .010
Thomas Laxton (a)...	93	8	.193 ± .009	.017 ± .012	1.4
Thomas Laxton (b)...	93	7	.187 ± .005	.023 ± .011	2.2
Laxtonian.....	93	8	.197 ± .004	.013 ± .010	1.2
Dwarf Alderman.....	93	8	.233 ± .006	.023 ± .011	2.1
Laxton's Progress.....	92	8	.145 ± .003	.065 ± .010	6.2
Improved Gradus.....	91	8	.294 ± .011	.084 ± .012	6.7
Rogers 95.....	89	8	.160 ± .005	.050 ± .011	4.7
Asgrow 40.....	89	8	.113 ± .003	.097 ± .011	9.1
Duplex.....	88	8	.193 ± .003	.017 ± .010	1.6

¹ See footnote to Table 2 for explanation of R.

ever, there was no way of knowing the size characteristic of maturity for the particular variety and therefore peas of the same size, No. 6, from all varieties, were selected and comparison made on that basis. The results are summarized (Table 3).

Compared with the Telephone variety, Thomas Laxton, Laxtonian, Dwarf Alderman, and Duplex varieties showed no significant difference in ascorbic acid content; Improved Gradus were definitely richer in ascorbic acid and Laxton's Progress, Rogers 95, and Asgrow 40 all had significantly less of the vitamin. These values might be quite different if it were possible to compare all varieties at the same stage of maturity.

Mack, Tressler, and King (1936) report that large, fresh Thomas Laxton peas had .26 mg. ascorbic acid per gram and Dwarf Alder-

man, large, .21 mg. per gram; the frozen-pack peas in this investigation, size No. 6, are comparable in ascorbic content with the fresh peas reported by these investigators. Soil and climate probably are factors in influencing the ascorbic acid content of peas grown in different parts of the country.

Thomas Laxton (a) (Table 3) were grown on plots receiving fertilizer treatment of 200 pounds superphosphate per acre, and Thomas Laxton (b) were grown on comparable plots but without added soil fertilizer. In this instance, no significant difference in ascorbic acid content of the peas was found.

SUMMARY

Measurement of the ascorbic acid content of garden peas preserved by the frozen-pack method in hermetically sealed containers showed comparable values by animal assay and by titration with 2,6 dichlorophenolindophenol.

There was no difference in ascorbic acid content of Telephone peas scalded for one minute in steam or in water at 99°C. (210.2°F.). Scalding in water for three minutes at 99°C., and for two, four, and six minutes at 71°C. (159.8°F.) resulted in a lower content of ascorbic acid.

Smaller peas were found to be higher in ascorbic acid per unit of weight than larger peas of the same variety; the seed coat was also found to contain more ascorbic acid per gram than the cotyledon.

Thawed peas removed from the original hermetically sealed containers and allowed to stand at room temperature lost 16 per cent of their ascorbic acid in 30 minutes, and 27 per cent in one hour; 25 per cent was lost on standing 24 hours in a refrigerator at 4.5°C. (40°F.).

Varietal differences were found to influence the ascorbic acid content of peas.

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IMPROVED PROCEDURES IN DETERMINATION OF ALDEHYDES IN DISTILLED ALCOHOLIC LIQUORS WITH SCHIFF'S REAGENT¹

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INTRODUCTION

Although it has been in use for 40 years or more, Girard and Cuniassé (1899), and is the official method of the A.O.A.C. (1935), the colorimetric determination of aldehydes in alcoholic liquors by means of Schiff's fuchsin-sulfur dioxide reagent has received adverse criticisms, according to Beyer (1936), and still presents certain difficulties in application. It is the purpose of the present paper to give methods whereby these difficulties may be in part overcome.

One fundamental difficulty is that, as usually conducted, a Schiff's reagent of undetermined sensitivity is used to determine the amount of aldehyde in unknown solutions diluted with a presumably aldehyde-free alcohol. Thus two unknowns are used in determining a third unknown. A colorless blank is usually regarded as indicating that the reagents are satisfactory, whereas it may merely indicate that the Schiff's reagent is relatively insensitive owing to excess SO_2 . This has apparently led in some cases to the publication of analyses in which samples of liquor are reported as containing zero aldehyde, a very dubious result when it is considered that even highly rectified alcohol, such as cologne spirit, almost invariably contains easily detectable amounts of aldehydes and must be specially purified for use in the aldehyde determination. Color in the blank is usually considered to indicate that the aldehyde has not been sufficiently removed from the alcohol, while in fact it may merely indicate an over-sensitive Schiff's reagent. The latter is particularly the case when a more deeply colored layer appears at the surface of the mixtures in the Nessler tubes, probably due to the action of atmospheric oxygen. By testing the Schiff's reagent, however, against an alcohol blank and a standard solution containing a small but known amount of aldehyde diluted with the same alcohol, the difficulty may be partly overcome. A colorless blank, together with a light pink color in the standard solution, indicates that the alcohol and the reagent are so adjusted to each other that the presence of a very small additional amount of aldehyde gives a positive test.

¹ Contribution No. 3 of the Destilería Serrallés.

Beyer (1936) claims that alcohol (cologne spirits, U.S.P.) giving a 45-minute permanganate test, needs no treatment for removal of aldehydes, but our own experience using a decolorized, highly sensitive Schiff's reagent has not confirmed this view.

PREPARATION OF COLORLESS FUCHSIN-SULFITE REAGENT

A modification of Woodman's (1931) method of preparation yields a much improved reagent. Dissolve .500 gram of good-quality basic fuchsin in 500 c.c. of warm water, and pass in sulfur dioxide gas until the weight has increased by five grams. Make to one liter. Although the reagent should now be colorless, it will usually be found to retain a yellow or orange color, which seriously obscures the light pink color produced in determinations on products low in aldehydes. This yellow color is apparently due to some impurity which is found in the basic fuchsin of even the best manufacturers. To remove it, add one gram of decolorizing carbon (the Suchar used in sugar decolorization is good), agitate, and filter. During filtration, the filter should be covered with a watch glass to prevent undue contact with the air. One treatment with carbon will sometimes yield a perfectly colorless "water-white" reagent, but if a trace of the discoloration remains, the carbon treatment should be repeated. The reagent so prepared is extremely stable, and if stored in a well-stoppered container will give satisfactory results even after five months or more at room temperature. In laboratories where many determinations are made, it is convenient to prepare double lots (one gram of fuchsin to give two liters of reagent) eliminating the preparation of reagent at more frequent intervals.

ADJUSTMENT OF FUCHSIN-SULFITE REAGENT FOR SENSITIVITY

The Schiff's reagent as prepared above will often be satisfactory without further treatment, but owing to the fact that fuchsin is seldom if ever 100 per cent in true dye content and varies in strength in different lots, an adjustment for sensitivity is frequently necessary. For a reasonable limit of sensitivity, the amount of SO_2 in the reagent should be such that a perfectly distinct, although light, pink color will be produced with .20 c.c. of the dilute standard acetaldehyde as given in the A.O.A.C. method (corresponding to .04 mgm. of acetaldehyde), while a blank determination on aldehyde-free alcohol is colorless or at most gives a much lighter pink than .20 c.c. of standard solution.

If more than a trace of pink is produced in the blank, more SO_2 should be passed into the Schiff's reagent for a minute or so and the process repeated if necessary, until a carefully prepared aldehyde-

free alcohol gives no color. Care should be taken that so much is not added that .20 c.c. of dilute standard acetaldehyde solution will not give an appreciable pink. If too much SO_2 has been passed in, small amounts (say .05 gm.) of basic fuchsin should be dissolved in the reagent (with carbon treatment if necessary) until the reagent is of satisfactory sensitivity.

A colorless Schiff's reagent of this type, adjusted to proper sensitivity for the conditions under which it is to be used, would no doubt be extremely useful also in qualitative organic chemistry, to replace the discolored and somewhat unsatisfactory solutions commonly employed.

PREPARATION OF ALDEHYDE-FREE ALCOHOL

A high-quality 95-per cent alcohol giving a permanganate (Barbet) test of 45 minutes or more by the method of Allen (1923), can usually be satisfactorily freed of aldehydes by the conventional method of refluxing for eight hours with five grams of meta-phenylenediamine hydrochloride per liter and distilling, rejecting the first 100 c.c., and not distilling the last 200 c.c. Small granules of unglazed tile should be added to reduce bumping. Under these conditions, preliminary distillation over NaOH or KOH, A.O.A.C. (1935), or over Ag_2O , Woodman (1931), is not necessary. The preparation of a second and a third lot of aldehyde-free alcohol, by bringing the "bottoms" of the first distillation to 1,000 c.c. by addition of fresh alcohol, refluxing and distilling as before, is possible; but it is not advisable to repeat the process more than three times, especially if more than a few days elapse between distillations, since the m-phenylenediamine-HCl (although present in great excess in relation to the amounts of aldehyde found in a good grade of alcohol) appears to slowly deteriorate upon standing in alcohol solution and to lose much of its ability to combine with aldehydes.

A slight modification of the method of Stout and Schuette (1933) gives an aldehyde-free alcohol very nearly as satisfactory as that made by treatment with the amine. Pour one liter of good-quality alcohol upon 10 grams each of KOH and Al turnings. Reflux for three hours. Distill, rejecting the first 100 c.c., which contains a trace of aldehyde, and leaving the last 100 c.c. in the flask at the close of distillation. Alcohol prepared by this method gave an almost completely colorless blank test, the excessively faint pink produced being noticeable only by comparison with alcohol freshly distilled off the amine. This method has the advantage that a relatively short refluxing time is required, and that the distillation proceeds much more smoothly than the m-phenylenediamine-HCl distillation, owing to the evolution of H_2 from the KOH and Al, which serves to prevent

bumping. It has the disadvantage that after a day or so considerable resinification occurs in the undistilled alcohol remaining in the flask, so that when more alcohol is added, refluxed, and distilled as before, the distillate contains amounts of aldehyde which render it quite unfit for use. Accordingly, the same reagents cannot be used for preparing a second lot of aldehyde-free alcohol unless, perhaps, the second lot of alcohol is added immediately after the end of the first distillation, and the second refluxing and distillation are conducted immediately.

Aldehyde-free alcohol at 95 per cent, made by treatment with the amine, will keep for at least four weeks in glass-stoppered bottles at about 10°C. (50°F.), but older samples will have generally formed enough aldehyde to render them undesirable for use. After dilution to 50 per cent, the solution should be used the *same* day that it is prepared. Alcohol treated by the modified Stout-Schuette process is apparently a little less stable in storage and may deteriorate more rapidly.

STABILITY OF ACETALDEHYDE STANDARD SOLUTION

The dilute standard acetaldehyde solution should be used the *same* day that it is prepared by dilution from the concentrated solution, since tests against day-old solutions sometimes show a loss in strength even if the solution has been stored in a closed container in the refrigerator.

The strong standard acetaldehyde solution has been kept without loss of strength for 10 months in well-closed containers at about 5°C. (41°F.) in an electric refrigerator, but another standard made from the aldehyde-ammonia of a different manufacturer and similarly preserved, lost considerable strength after eight months. Three months is perhaps a reasonable limit for storage at low temperature. The standard solution should be neutral or *faintly* acid to "neutral" litmus paper. Solutions which gradually darkened and lost considerable strength in low-temperature storage were found to be alkaline to litmus paper. Although the required quantity of standardized alcoholic H_2SO_4 had been added to the alcoholic solution of aldehyde-ammonia, apparently not all of the ammonia had been neutralized. This apparently caused slow resinification. Accordingly, the mixture should be tested after the alcoholic H_2SO_4 has been added; and, if alkaline, should be neutralized or made slightly acid to litmus paper before making to 100-c.c. volume. In filtering, the filter paper should be covered with a watch glass to minimize evaporation losses of the highly volatile acetaldehyde. It is a serious objection to the Schiff method as it exists at present, that its basic standard is based upon a compound so subject to deterioration as aldehyde-ammonia, which

resinifies with time and presumably loses strength to an undetermined degree. The products of two different manufacturers, kept in the original containers at a rather high average room temperature, showed signs of spoilage after a few months and after two years had decomposed into a brown, amorphous, semi-pasty mass.

Although the products of three different manufacturers were tried, no aldehyde-ammonia was found which completely lost its brownish color even after several washings with anhydrous ether. In fact the ether washing seems to be of little or no value and might well be eliminated. Eastman Kodak Company's aldehyde-ammonia, No. T560, was found to be the most satisfactory from the standpoint of low color and low apparent resin content. In hot weather the compound appears to resinify more rapidly than in cool weather and hence had best be preserved in an electric refrigerator. To reduce condensation when removed for use, it is well to keep the bottle containing the compound in some larger container and to allow the whole to come to room temperature before removing any aldehyde-ammonia for weighing.

The alcoholic H_2SO_4 appears to keep well at low temperatures. It is convenient to prepare a considerable quantity of it by diluting three c.c. of concentrated H_2SO_4 to 300 c.c. with aldehyde-free 95-per cent alcohol and standardizing the solution by titration against standard NaOH solution.

METHOD OF CONDUCTING DETERMINATION

The determination should be conducted at $15^\circ\text{C}.$ ($59^\circ\text{F}.$), as prescribed in the A.O.A.C. method (1935). The Schiff's reagent should be kept in the refrigerator until immediately before use. It does not matter if the reagent is colder than $15^\circ\text{C}.$, but higher temperatures will give a greater development of color leading to erroneous results. After adding the Schiff's reagent, the contents of each tube should be mixed by placing the thumb over the mouth of the tube and inverting once before returning to the bath, avoiding undue agitation. Incomplete mixing may cause uneven colorations and a ruined determination. Stoppering the tubes after mixing is probably unnecessary. A considerable saving of reagents will be effected if the determinations are made on about half the usual scale, diluting only to 25 c.c. with 50-per cent aldehyde-free alcohol and using only 10 c.c. of Schiff's reagent. Nessler tubes of 50-c.c. capacity may be used. Tubes should be compared against a diffusely lighted, white background. To eliminate sidelights, they may be placed side by side in a mailing tube open at both ends. It is more convenient to work with standards containing low concentrations of aldehyde (one c.c. or less of dilute

standard solution) than with higher concentrations which give darker colors more difficult to match. If accurate fractional pipettes are used, little if any loss in accuracy will result.

SUMMARY

An entirely colorless Schiff's reagent, sensitive to .04 mgm. of acetaldehyde in 25 to 50 c.c. of 50-per cent alcohol and stable for at least five months at room temperature, may be prepared by treatment with decolorizing carbon and careful adjustment of the amount of sulfur dioxide.

The A.O.A.C. strong, standard, acetaldehyde solution will keep for three months or more at 5°C. (41°F.), without loss of strength, if neutral or faintly acid to litmus paper. Traces of free ammonia cause it to darken in storage and become unfit for use. The diluted solution should be used the *same* day that it is prepared.

Alcohol with a 45-minute KMnO_4 test time can be satisfactorily freed of aldehydes by the usual method of refluxing with m-phenylenediamine-HCl for eight hours and distilling, without preliminary NaOH or Ag_2O treatment. The same reagent can be used two times more by addition of more alcohol and repeating the process. The aldehyde-free alcohol will keep for four weeks or longer if stored at 10°C. (50°F.).

A satisfactory aldehyde-free alcohol can also be made by refluxing one liter for three hours with 10 grams each of KOH and Al turnings, and distilling, rejecting the first and last 100 c.c., but this alcohol is apparently somewhat less stable in storage.

A careful study has been made of precautions to be observed in preparing, standardizing, and storing the reagents and in conducting the determination.

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VARIATIONS IN SAMPLING BEEF AND PORK ROASTS FOR PRESS-FLUID INVESTIGATIONS¹

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Sampling is a pertinent and often perplexing problem in all meat research but particularly so for press-fluid determinations. The study reported in this paper was initiated, therefore, to gather more data concerning the effect of method of sampling upon amount of press fluid obtained. The term "press fluid" was used by Child and Baldelli (1934) to designate the fluid consisting of moisture, soluble material, and colloidal fraction pressed from muscle by the pressometer. The amounts of press fluid obtained under standard conditions were analyzed statistically to determine the degree of variation between samples taken as follows:

- (1) Samples from comparable slices within the roast (beef and pork).
- (2) Samples from successive slices proceeding from the center of the roast (beef and pork);
- (3) Samples from different cuts (pork).

In addition, the correlation between press fluid and cooking losses was obtained, since cooking losses have been used as one of the bases for comparing methods of cooking meat.

EXPERIMENTAL PROCEDURE

Beef: Beef of choice quality, aged two weeks, and taken from animals as nearly uniform as possible was procured from a packing house. The semitendinosus muscle was used. The beef experiments constitute Series I to III.

Pork: Pork loins aged four days, averaging 10 to 12 pounds, were divided into the following roasts: (1) the shoulder, containing five ribs (Series V); (2) the center five thoracic vertebrae (Series IV and VI); and (3) the loin end with one thoracic and four lumbar vertebrae (Series VII).

Cooking: Beef roasts were placed on a rack in a sheet-iron dripping pan and cooked in an electrically controlled oven at 125°C. (257°F.) to an internal temperature of 53°C. (136°F.) and were

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² Deceased July 10, 1938.

cooled to 40°C.(104°F.) before sampling. Pork roasts were cooked in a sheet-iron dripping pan without a rack at 150°C.(302°F.) to an internal temperature of 84°C.(183°F.).

Cutting Slices: The roasts were halved with a thin-bladed, sharp knife, as shown (Fig. 1), considering the insertion point of the thermometer as the center of the roast, X; and successive half-inch slices, A, B, and C, were cut from one half and A', B', and C' from the other. A and A', B and B', and C and C' are considered comparable slices within a roast.

Press Fluid: Duplicate samples at comparable positions near the center of each slice were taken by means of a cylindrical borer 1.25 cm. in diameter. The weighed sample was wrapped in an unsized, weighed, filter cloth and placed on the tray of a pressometer (an

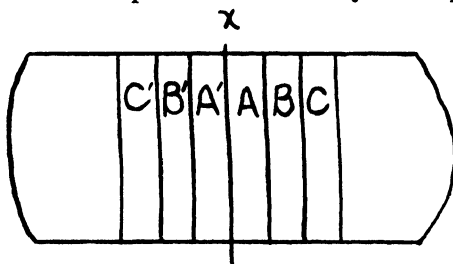


FIG. 1. Longitudinal section of roast showing location of slices compared.

electrically controlled apparatus for pressing fluid from muscle) under a pressure of 250 pounds per square inch for 10 minutes. The quantity of press fluid was estimated by taking the difference in weight of the samples before and after pressing and presenting the results in terms of percentage by weight. Details of the method are described by Child and Baldelli (1934).

In an additional group of experiments with pork loins (Series V to VII) designed to investigate differences between center, shoulder, and loin-end cuts, the loins were cut into the three parts and each was roasted separately. In this paper, these will be designated as "cuts" of loin. These cuts were not sampled by division into six slices but, rather, two duplicate samples for pressing were cut from a slice taken from the middle of each cut (comparable to slice A).

RESULTS AND DISCUSSION

Analysis of variance, according to Fisher (1932) and Snedecor (1934), applied to the original data from the 18 beef roasts (Table 1) revealed that there was a highly significant difference (greater than the one-per cent point)³ between roasts and also between the six

³ As shown by Snedecor's "F" test.

slices from a roast, using the interaction as the error. The interaction between roasts and slices was found to be significantly greater ($F = 2.25$) than the variance owing to differences between duplicate samples taken from the same slice. Further analysis (Table 2) in-

TABLE 1

Analysis of Variance for Press Fluid in Slices Varying in Position in Semitendinosus Muscle of Beef, Cooked to 58°C. at 125°C. (Series III)

Variation due to:	Degrees of freedom	Sum of squares	Mean square	F
Roasts.....	17	527.50	31.03	9.89 ¹
Slices.....	5	634.60	126.92	40.47 ¹
Interaction.....	85	266.59	3.14
Samples.....	107	1,428.69
Duplicates within samples.....	108	150.38	1.39
Total.....	215	1,579.07

¹ Exceeds the one-per cent value for F.

TABLE 2

Mean Percentage Press Fluid in Slices Varying in Position in Semitendinosus Muscle of Beef Cooked to 58°C. at 125°C. (Series III)

Comparison of comparable slices	Comparison of different slices	Comparison of halves of roasts
Means of: A = 58.4 A' = 58.3 B = 56.7 B' = 56.1 C = 54.4 C' = 54.0	Means of: A + A' = 58.4 B + B' = 56.4 C + C' = 54.2	Means of: A + B + C = 56.5 A' + B' + C' = 56.2
S.E. _{diff} = .59	.42	.34

indicated that the means of the 18 samples from comparable slices from the beef roasts (i.e., \bar{A} vs. \bar{A}' , \bar{B} vs. \bar{B}' , and \bar{C} vs. \bar{C}') were not significantly different when compared with the standard error of the difference. An additional comparison of halves of roasts (i.e., $\frac{1}{2} [\bar{A} + \bar{B} + \bar{C}]$ vs. $\frac{1}{2} [\bar{A}' + \bar{B}' + \bar{C}']$) showed no significant difference, the difference being less than the five-per cent level. A high degree of significance, however, was found between the means of $(\bar{A} + \bar{A}')$, $(\bar{B} + \bar{B}')$, and $(\bar{C} + \bar{C}')$. This is especially interesting when it is noted that there is likewise a steady decline in press fluid, proceeding from slice A to slice C, indicating that the higher in-

ternal temperatures attained by the ends cause greater cooking losses. Child and Fogarty (1935) also found an inverse relationship between percentage of press fluid and total cooking losses in beef muscle heated to 75°C. (167°F.).

TABLE 3

Analysis of Variance for Press Fluid in Slices Varying in Position in Longissimus Dorsi Muscle of Pork Cooked to 84°C. at 150°C. (Series IV)

Variation due to:	Degrees of freedom	Sum of squares	Mean square	F
Roasts.....	17	2,352.45	138.38	40.29 ¹
Slices.....	5	50.01	10.00	2.91 ²
Interaction.....	85	291.94	3.43	
Samples.....	107	2,694.40
Duplicates within samples.....	108	355.81	3.29
Total.....	215	3,050.21

¹ Exceeds the one-per cent value for F. ² Exceeds the five per cent value for F.

The data on pork samples (Table 3), when treated by the analysis of variance, also showed a high significance between roasts. The variance of duplicates within samples was not significantly lower ($F = 1.14$) than the interaction between roasts and slices, but that between comparable slices must be considered as significant for it

TABLE 4

Mean Percentage Press Fluid in Slices Varying in Position in Longissimus Dorsi Muscle of Pork Cooked to 84°C. at 150°C. (Series IV)

Comparison of comparable slices	Comparison of different slices	Comparison of halves of roasts
Means of: A = 48.1 A' = 47.4 B = 46.9 B' = 46.7 C = 46.9 C' = 47.2	Means of: A + A' = 47.8 B + B' = 46.8 C + C' = 47.1	Means of: A + B + C = 47.3 A' + B' + C' = 47.1
S.E. _{diff} = .62	.44	.36

would have occurred by chance alone less than once in 20 times. These statistics are borne out by the calculations (Table 4). Here no significant difference between means of comparable slices or between halves of roasts could be found, but the difference between $\frac{1}{2}(\bar{A} + \bar{A}')$ and $\frac{1}{2}(\bar{B} + \bar{B}')$ was greater than twice the standard error of the difference. No significant difference was found between $\frac{1}{2}(\bar{A} + \bar{A}')$

and $\frac{1}{2}(\bar{C} + \bar{C}')$ or between $\frac{1}{2}(\bar{B} + \bar{B}')$ and $\frac{1}{2}(\bar{C} + \bar{C}')$, nor was there any gradient in the percentage of press fluid from A to C as found in the beef investigations. This is probably due to the fact that the pork roasts are cooked to a higher temperature than the beef.

TABLE 5

Analysis of Variance of Mean Percentage Press Fluid in Center, Shoulder and Loin Ends of Pork Loin Cooked to 84°C. at 150°C. (Series V-VII)

Variation due to:	Degrees of freedom	Sum of squares	Mean square	F
Roasts.....	19	790.80	41.62	1.60
Cuts.....	2	349.79	174.89	6.74 ¹
Interaction.....	38	985.37	25.93
Samples.....	59	2,125.96
Duplicates within samples.....	60	383.30	6.39
Total.....	119	2,509.26

¹ Exceeds the one-per cent value for F.

Highly significant differences between the press fluid from the three cuts of the pork loins (Table 5, based on roasts from 10 right and 10 left loins) were found, upon application of the analysis of variance. In this case, however, only one slice was taken from each roast (Slice A), whereas in the previous study (Table 3) several slices were taken from the same roast. σ due to the experimental

TABLE 6

Mean Percentage Press Fluid in Center, Shoulder, and Loin Ends of Pork Loin Cooked to 84°C. at 150°C. (Series V-VII)

Cut ¹	Mean raw weight per roast	Press fluid per roast
	kg.	pct.
Shoulder loin.....	1.525	48.8
Center loin.....	1.140	52.0
Loin end.....	2.579	48.1
S.E. _{diff} = 1.61

¹ Cuts from 10 right and 10 left loins.

error was 5.09 in this study, whereas in the beef and pork studies (Tables 1 and 3) it was 1.77 and 1.85, respectively. In this experiment, however, no significant difference in press fluid was found between the roasts. It was found on further analysis (Table 6) that although the difference between the mean percentage fluid from shoulder and loin end was not significant, the center cut was sig-

TABLE 7

Mean Percentage Press Fluid and Cooking Losses in Semitendinosus Beef Muscle Cooked to 58°C. at 125°C., and Longissimus Dorsi Muscle of Pork Cooked to 84°C. at 150°C.

Series	Cut	Number of roasts	Mean raw weight per roast	Mean press fluid per roast		Mean cooking losses						Correlation coefficients between press fluid and total cooking losses	
				pct.	σ	Evaporation		Dripping		Total			
						pct.	σ	pct.	σ	pct.	σ	pct.	σ
I	Beef.....	12	0.691	54.5	2.38	7.4	1.46	1.3	0.95	8.7	2.18	— .4287	
II	Beef.....	18	0.753	55.5	1.57	7.8	1.22	1.4	0.79	8.2	1.90	+ .2723	
III	Beef.....	18	0.996	56.3	1.62	6.8	0.75	1.4	0.74	8.2	1.24	— .2915	
IV	Pork (center).....	18	1.133	47.2	3.12	8.9	1.20	7.4	1.81	16.2	2.40	— .7150	
V	Pork (center) ¹	20	1.140	52.0	3.19	8.0	1.64	7.0	2.72	15.0	5.13	— .7441	
VI	Pork (shoulder) ¹	20	1.525	48.8	3.76	13.0	2.67	4.2	1.62	17.2	3.12	— .5313	
VII	Pork (loin end).....	20	2.579	48.1	4.60	11.7	3.06	8.2	2.02	19.9	3.95	— .6118	

¹ Cuts from 10 right and 10 left loins.

nificantly higher in press fluid than either of the others. It should be noted (Table 5) that variance owing to duplicate samples within roasts was significantly lower ($F = 4.06$) than the interaction.

The correlation coefficient, according to Fisher (1932), between press fluid and total cooking losses is presented (last column of Table 7) for both beef and pork investigations. From these calculations the coefficient of correlation between press fluid and total cooking losses in semitendinosus beef roasts cooked to $58^{\circ}\text{C}.$ ($136^{\circ}\text{F}.$) is not significantly different from zero. This is in accord with the work of Child and Fogarty (1935) when using the same temperature. Their results show, however, that when the internal temperature is $75^{\circ}\text{C}.$ ($167^{\circ}\text{F}.$) there is a significant negative correlation between press fluid and total cooking losses. In the pork investigations, Series IV, V, and VII exhibit a highly significant, and Series VI a significant, negative correlation⁴ between these variables. These results are in agreement with the work of Meigs (1909) and McCance and Shipp (1933) who state that muscle tissue shortens without a change in volume or loss of weight when heated to $40^{\circ}\text{C}.$ ($104^{\circ}\text{F}.$) but that at temperatures above $60^{\circ}\text{C}.$ ($140^{\circ}\text{F}.$) there occurs a loss of weight caused by increased shrinkage.

SUMMARY

From this investigation the following observations on press fluid from semitendinosus beef muscle, cooked to $58^{\circ}\text{C}.$ ($136^{\circ}\text{F}.$) at $125^{\circ}\text{C}.$ ($257^{\circ}\text{F}.$), and longissimus dorsi muscle of pork, cooked to $84^{\circ}\text{C}.$ ($183^{\circ}\text{F}.$) at $150^{\circ}\text{C}.$ ($302^{\circ}\text{F}.$), can be made on the basis of statistical analysis:

1. The quantity of press fluid from comparable slices of beef and of pork taken at equal distances from each side of the center of the roast did not vary significantly, nor was there a significant difference between halves of roasts.

2. Analysis of variance between successive slices ($A \rightarrow B \rightarrow C$) proceeding from the center of the roast showed that these were significantly different from each other. It was found that in the beef experiments press fluid decreased progressively from the center to the ends of the roast ($A > B > C$).

In the pork investigation there was no gradient in the percentage of press fluid from A to C, probably due to the fact that the pork roasts were cooked to a higher temperature than the beef.

3. Press-fluid percentages from the loin end and shoulder cuts of pork were not significantly different but both cuts gave amounts which were significantly lower than the center cut.

⁴ Taking the one-per cent level as highly significant and the five-per cent level as significant, Fisher (1932).

4. Significant negative coefficients of correlation between cooking losses and amounts of press fluid were obtained in the pork experiments. No such association was found with beef.

The authors wish to express appreciation to Prof. F. R. Immer for his helpful suggestions regarding the statistical analysis.

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ADHESION OF POTATO-TUBER CELLS AS INFLUENCED BY TEMPERATURE ¹

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The physical and chemical alterations which occur during the cooking of potato tubers have been investigated by a number of workers. The literature has been reviewed recently by Sweetman (1936), and a book on the subject has been published by Rathsack (1935). Much attention has been given to the composition of potatoes, particularly to the starch content and its effect upon mealiness and sogginess. It has been suggested by Coudon and Bussard (1897), Day (1909), Butler, Morrison, and Boll (1913), Cobb (1935), Sweetman (1936), and Sweetman and Dakin (1936) that a mealy potato results when the cells of the cooked tissue separate readily. Heretofore the ease and completeness of the separation of the cells was determined by subjective observation. This does not enable one to determine accurately small differences in degree of adhesion of the cells. The present paper reports some phases of a study on the effect of heat on the ease of separation of the tuber cells as determined by measurements of the tensile strength.

METHOD OF DETERMINING TENSILE STRENGTH OF POTATO TISSUE

Tensile strength is defined as the minimum longitudinal stress required to pull a section of potato-tuber tissue asunder. To determine the tensile strength of potato tissue, uniform potato slices approximately eight mm. thick were cut from the tubers by means of a small, motor-driven, circular, crosscut saw. The potato during the cutting was held in a traveling guide so that slices of uniform thickness were produced. Dumb-bell-shaped sections were cut crosswise of these slices using a brass punch cutter 3.2 cm. long and in the central narrow part .4 cm. long and .5 cm. wide. The enlarged ends

¹ In part condensed from a thesis presented by Catherine Personius to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1937.

The authors wish to acknowledge the assistance of Mr. Raphael Keith in the construction of the apparatus used in this investigation.

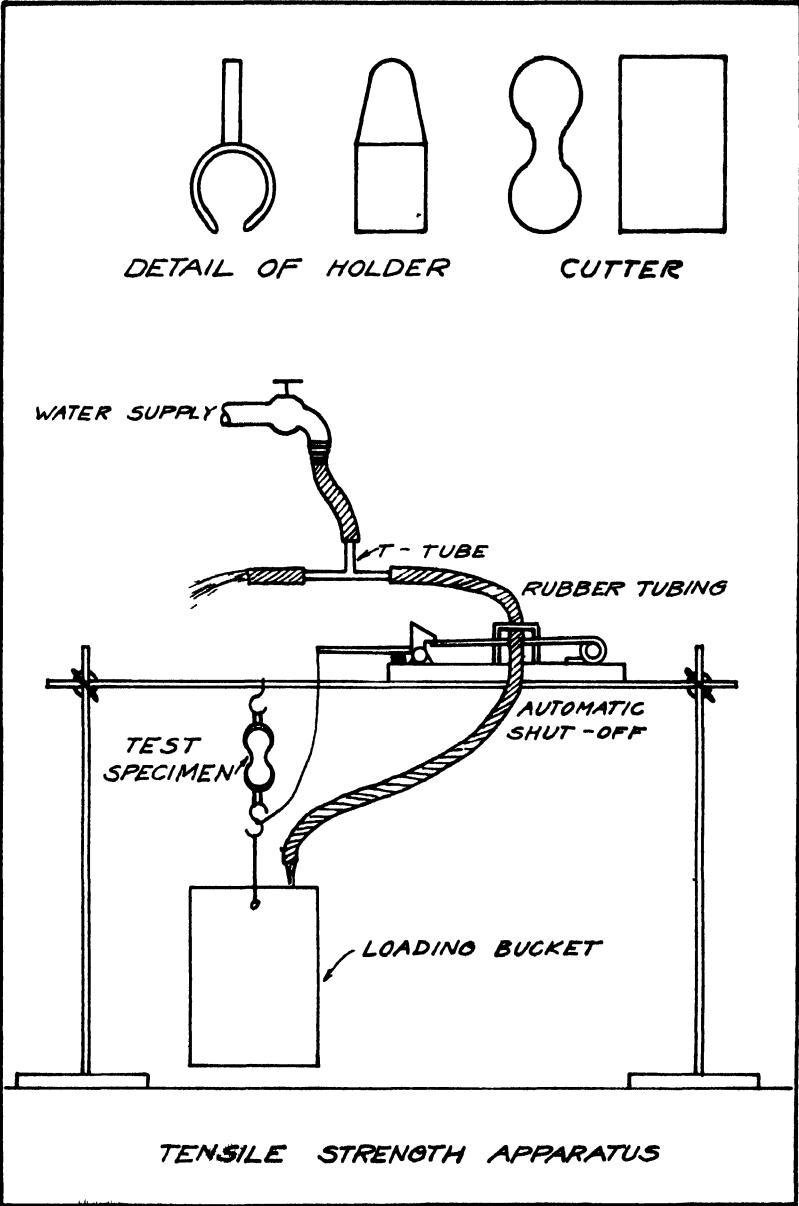


FIG. 1. Apparatus for determining the tensile strength of potato-tuber tissue.

of the sections were secured in close-fitting holders. By means of one of the holders each section was suspended from a horizontal bar; a container was hung from the other holder. By running water into this container a stress of increasing magnitude was applied until the section ruptured. By means of a spring device, water running into the container from the tap was automatically shut off at the instant of rupture. The weight required to rupture the section was determined by weighing the container and water. The dimensions of the ruptured surface were measured with calipers and the area calculated. The tensile strength of the tissue was expressed in kilograms per square centimeter ($\frac{\text{kg.}}{\text{sq. cm.}}$) required to produce the rupture.

The apparatus for determining tensile strength is illustrated (Fig. 1).

SAMPLING TUBER TISSUE

An extensive study was made of the method of cutting test pieces from the tuber. The tensile strength of test sections was not influenced by the size of tuber from which sections were cut. Similar

TABLE I
*Effect of Presence of Inner and Outer Medulla on Tensile Strength
of Potato Tissue*

(Expressed in kg. per sq. cm.)

Tuber No.	Predominantly inner medulla			Predominantly outer medulla		
	Highest	Lowest	Average	Highest	Lowest	Average
18	9.48	6.00	7.87(11) ¹	7.21	4.70	6.17(11)
19	7.86	5.64	7.00(8)	7.06	5.22	6.04(18)
20	9.05	5.74	7.30(8)	7.04	5.44	6.09(9)
21	7.40	5.63	6.52(6)	6.78	4.31	5.68(18)
22	7.18	6.16	6.82(6)	7.08	4.46	5.84(20)
23	7.80	5.43	6.53(13)	6.87	5.18	5.75(15)
24	7.95	6.33	7.03(11)	8.08	5.07	6.45(8)
25	7.94	6.15	7.22(9)	7.21	4.40	6.02(20)
26	8.67	6.87	7.68(6)	7.69	5.61	6.41(13)
27	7.43	5.47	6.39(11)	6.44	3.95	5.38(16)

¹ The numbers in parenthesis indicate the number of test sections.

results were obtained with slices cut lengthwise or transverse of the tuber and with sections cut lengthwise, crosswise, or diagonally of the slices. Neither the weight of the section nor the extensibility of the section under stress influenced the tensile-strength value. So far as could be observed in microscopic examinations of the tissue, no relationship existed between the tensile strength of the raw tubers and such physical characteristics of the tissue as size of the cells, thickness of the cell walls, amount of starch in each cell, or size of

TABLE 2

*Tensile-Strength Values Obtained by Using Different Methods of Selecting Test Sections*¹
(Expressed in kg. per sq. cm.)

Tuber No.	METHOD 1			METHOD 2				METHOD 3			METHOD 4		
	All alices			3 center, 2 end alices				3 center, 2 end alices			3 center alices		
	All sections with inner medulla	All sections with outer medulla	All sections	3 sections with inner medulla	7 sections with outer medulla	10 sections		5 sections with inner medulla	5 sections with outer medulla	10 sections	All sections with inner medulla	All sections with outer medulla	All sections
19	7.00	6.04	6.33	7.79	5.99	6.53		7.16	5.92	6.54	7.12	5.98	6.60
20	7.30	6.09	6.66	8.01	6.07	6.65		7.51	5.90	6.71	7.61	6.18	6.96
21	6.52	5.68	5.89	6.50	5.57	5.84		6.34	5.22	5.78	6.93	5.75	5.92
22	6.82	5.84	6.06	6.82	5.92	6.19		6.74	5.83	6.29	7.05	6.03	6.22
23	6.58	5.75	6.14	6.60	5.88	6.10		6.43	5.76	6.09	6.42	5.82	6.14
24	7.03	6.45	6.78	7.34	6.22	6.55		7.03	6.40	6.72	7.01	6.52	6.82
25	7.22	6.02	6.39	7.54	6.07	6.51		7.60	5.72	6.66	7.36	6.39	6.91
26	7.68	6.41	6.81	7.54	6.64	6.91		7.56	6.83	7.20	7.68	6.46	7.07
27	6.39	5.38	5.79	6.31	5.58	5.80		6.41	5.66	6.04	6.54	5.54	6.10
28	6.64	6.63	6.63	6.64	6.53	6.57		6.64	6.57	6.61	6.86	6.98	6.92
Av.	6.92	6.03	6.35	7.11	6.05	6.37		6.94	6.01	6.46	7.06	6.17	6.57

¹ Tubers were all from same lot.

the starch grains. No relationship was evident between the tensile strength of raw tubers and variety or texture.

When the central portion of the test section was predominantly inner medulla, higher tensile-strength values resulted. Therefore, after each test was made, the ruptured surface was examined and a record was made as to whether or not this central portion was predominantly of inner medulla or of outer medulla. Several hundred comparisons between the tensile strength of portions containing inner and outer medulla were made. The effect on tensile strength of the presence or absence of inner medulla, using ten test tubers, is shown (Table 1).

The lack of uniformity of the sections, owing to the presence or absence of inner medulla in the cut sections taken for testing, introduced a complication in sampling of the potato which required con-

TABLE 3
*Tensile-Strength Values of Potatoes of Different Varieties and
Different Textures*

(Expressed in kg. per sq. cm.)

Variety	Texture	Tuber No.				Average
		1	2	3	4	
Russet Burbank.....	Mealy	7.19	6.94	6.51	7.13	6.94
Smooth Rural.....	Mealy	6.55	6.68	7.44	6.86	6.88
Russet Rural ¹	Soggy	7.92 ²	6.88	6.99	7.31	7.28
Green Mountain ¹	Soggy	6.06	7.12	7.17	6.05	6.66

¹ Tubers were small. As many sections as could be obtained were tested. ² Over one-half of sections from this tuber contained inner medulla.

trol in order to obtain comparable results for tensile-strength values. A comparison of four different sampling procedures is given (Table 2). Taking the average values for all tubers examined by Method 1 as a standard, the deviations obtained with the other methods of sampling used are probably too small to be significant. Nevertheless agreement between Methods 1 and 2 is very close. As a result of this study and the data presented (Table 2) the second method was chosen. In this method the average value was obtained for ten test sections of a single tuber, three of the sections containing inner medulla, seven of the sections containing outer medulla. One section appearing to contain the largest proportion of inner medulla in its central area was selected from each of the three center slices of the tuber. One section appearing to contain either none or the least inner medulla was selected from each of the two end slices and the center slice of the tuber. Two sections without inner medulla were selected from each of the remaining center slices. Coudon and Bussard (1897)

found that the inner medulla of the potato comprised 17.38 per cent and the outer medulla 37.63 per cent. Glynne and Jackson (1919) give the values 19.7 and 36.9 per cent. This is not far from the ratio of three to seven used in our testing procedure.

VARIETY AND STORAGE

Some tensile-strength values for four varieties of potatoes, some soggy and others mealy, indicate (Table 3) that neither varietal nor texture differences influence the tensile strength of the raw tissue. Most of the work which follows was done on potatoes of the White

TABLE 4

Tensile-Strength Values of Potatoes Stored for Varying Periods of Time in Different Atmospheres and at Various Temperatures

(Expressed in kg. per sq. cm.)

Days	22°C. (71.6°F.)			10°C. (50°F.)			3°C. (37.4°F.)		
	Air	CO ₂	CO ₂ free	Air	CO ₂	CO ₂ free	Air	CO ₂	CO ₂ free
2.7	6.86	6.98	7.29
3.8	6.06	7.13	6.89	6.29	7.16
4.7	7.09	6.70	7.14	7.08	7.22	7.21	6.84	6.98
5.7	6.41	7.11	6.45	6.85	6.47	6.34
6.7	6.38	6.19	6.04
7 or 8	6.74	6.49	6.59	6.54	6.81	7.18	5.60 ¹	7.20	6.69
14 or 15	7.20	7.47	7.68	7.58	6.70	6.67	6.06 ¹	6.27 ¹	5.65 ¹
21 or 22	6.96	7.26	7.01	6.80	7.12	7.30	6.51 ¹	6.18 ¹	6.17 ¹
28	7.21	7.00	7.32	7.11	7.31	7.26	5.42 ¹	6.80 ¹	7.51 ¹
35 or 42	7.13	7.32	6.53	6.52	7.72	7.62	6.97 ¹	6.51
35 or 42	7.04	7.00	6.74	6.99	6.88	7.31 ¹	7.05	6.55
58 or 60	6.59	7.56	6.41	8.26	6.82	4.77 ¹	7.32	7.01 ¹
58 or 60	5.95	6.55	6.38	7.45	7.59	6.81 ¹	6.13 ¹	6.22

¹ Raw tuber showed appreciable discoloration throughout.

Globe variety of the Smooth Rural type. From the effect on tensile strength of several of the variables which potatoes might encounter on storage (Table 4) we conclude that the conditions under which the potatoes are stored exert little influence on the tensile strength of the raw potato tissue. However, final conclusions as to the influence of variety, texture, and storage must await further studies.

EFFECT OF TEMPERATURE

The relationship between the degree of doneness of a cooked potato and its tensile strength was determined. In the case of the cooked potato the circular saw could not be used, and it was necessary to cut the test slices by hand. Data show that cooked tubers, ones that could be readily mashed with a fork, gave tensile strength values of

.7 kg. per sq. cm. or less (Table 5). Usually the value was .5 kg. per sq. cm. or lower.

The effect of various degrees of heating on the tensile strength of potato tubers was studied in order to determine the critical temperature involved in reducing the tensile strength of raw-potato tissue to values like those characteristically obtained under normal conditions of cooking. The procedure used was to hold potato tubers at a series of constant temperatures and to determine the tensile strength of the tubers after various intervals of time. The potato tubers were held immersed in water. In order to prevent any exchange of water or salts between the tuber and the water bath, each tuber was carefully covered with rubber paint. Experiments were carried out in

TABLE 5
Tensile-Strength Values of Cooked or Nearly Cooked Tubers

Tuber No.	Kg per sq. cm.	Remarks
53	1.69	Parts of tuber can be mashed with fork.
55	1.23	Almost tender to fork.
59	0.94	Slightly resistant to mashing.
57	0.91	Almost tender.
61	0.80	Slightly resistant to mashing.
60	0.70	Mashes quite readily.
56	0.50	Seems like cooked tuber.
58	0.41	Mashes quite readily.
71	0.31	Mashes readily.
73	0.30	Mashes readily.
72	0.29	Mashes readily.
70	0.25	Mashes readily.

which thermocouples were introduced into tubers, and the time required for the center of a tuber to reach the temperature of the water was determined. It was found that four to six hours were required for the interior of the tuber to reach the temperature of the surrounding water. The effect of various heat treatments (Fig. 2) shows that no appreciable reduction in tensile strength of potato tissue occurs during 48 hours at temperatures of 50°C.(122°F.) or below. At higher temperatures the rate of reduction in tensile strength increases with the temperature. It is interesting to note that depending upon the temperature, the tensile strength decreases to a relatively constant value and this constant value is lower, the higher the temperature.

Microscopic observations on the condition of the starch were made in all cases. At temperatures up to 63°C.(145.4°F.) the starch grains were not greatly swollen. After 48 hours at temperatures above 67°C.

in which $^{\circ}\text{C.}$ is the temperature of holding. This equation yields values indicating that the tensile strength would decrease .24 kg. per sq. cm. in 100 days at 9°C. (48.2°F.) and 23.5 kg. per sq. cm. in one hour at 100°C. (212°F.). Therefore, at 100°C. , cooking would be complete in about 15 minutes in so far as ease of separation of the cells is concerned. These values extrapolated by means of the equation are in harmony with the facts of storage and cooking time.

At each temperature the tensile strength decreases to a constant value the exact magnitude of which depends upon the temperature

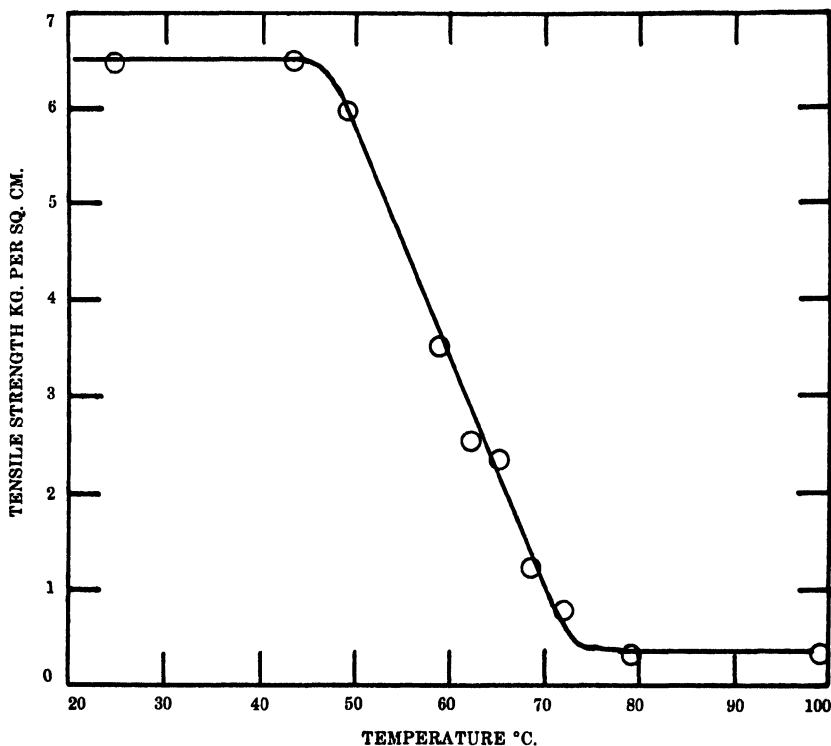


FIG. 4. Minimum values for the tensile strength of potato-tuber tissue obtained after holding at various temperatures. (See Fig. 2.)

(Fig. 2). The constant values which were attained at each temperature were plotted against the temperature and are shown (Fig. 4). These data show that weakening of the cementing material between the cells takes place between the temperatures of 45 and 75°C. (113 and 167°F.). The weakening of the cementing material goes just so far at each temperature within this range and then stops. This effect was not anticipated. It was expected that if the temperature were raised to a value which would weaken the cementing material apprecia-

bly, then by holding at this temperature for a sufficient time the weakening of the cementing material would continue until values essentially the same as those obtained at a higher temperature in a much shorter time would be reached. It seems evident, therefore, that within the temperature range of 45 to 75°C., at each temperature the weakening of the cementing material is carried to a definite point and then stops, and in order to weaken the cementing material further the temperature must be further increased. The complete weakening of the cementing material can be accomplished at 75°C. and, in so far as ease of separation of the cells is concerned, maintenance at higher temperatures can produce no additional effect but only attain the weakening of the cementing material in a shorter time.

A definite weakening of cementing material between the cells is brought about at temperatures below the gelatinization temperature of the starch. Consequently, cell separation and gelatinization are independent.

CONCLUSIONS

1. The ease of separation and the weakening of the cementing material between the cells of the potato tuber can be determined by measurements of tensile strength.

2. The tensile strength of the raw potato-tuber tissue is influenced only slightly, if at all, by variety or ordinary storage.

3. Inner medulla of the raw tuber possesses a higher tensile strength than does outer medulla.

4. The effect of temperature on the portion of the time-tensile strength curve showing a uniform decrease can be expressed by the following equation: log decrease in tensile strength in kg. per sq. cm. per hour = $(.059) ^\circ\text{C.} - 4.53$.

5. Within the temperature range of 45 to 75°C. (113 to 167°F.), at each temperature the weakening of the cementing material is carried to a definite point and then stops, and in order to weaken the cementing material further the temperature must be raised.

6. Complete weakening of the cementing material can be brought about by holding at 75°C., at which temperature tensile-strength values comparable to those of the fully cooked potato tissue are obtained.

7. No difference was noted between soggy and mealy potatoes in the adhesion of cells of the raw or cooked tissue.

8. Separation of the cells is not dependent upon starch gelatinization.

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PERMEABILITY OF POTATO-TUBER TISSUE AS INFLUENCED BY HEAT ¹

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The effect of heat on the adhesion of potato-tuber cells has been studied by Personius and Sharp (1938). The present article reports a study of the possibility of a relation between the loss of adhesion between the cells, starch swelling, and alterations in the permeability of the tuber tissue. Alterations in the electrical resistance of sections of tissue were used as an indication of changes in tissue permeability. The determination of electrical resistance also served as a method of investigating the possibility that in the final stages of cooking, separation of the tissue cells might occur with the formation of intercellular air spaces.

The conductance of plant tissue depends largely on the electrolytes dissolved in the cell sap. The membranes and protoplasm of the normal living cells offer resistance to passage of the current in proportion to the extent to which they hinder the migration of the ions on which the electrical conductivity depends. Any treatment which affects the permeability of the cell membranes and protoplasm would result in corresponding changes in resistance of the tissue, Osterhout (1922).

APPARATUS AND METHOD

The conductivity cell was made of a piece of Pyrex glass tubing 5.5 cm. in length and 1.6 cm. in internal diameter. Small vent tubes were sealed 1.5 cm. from each end of the cell and a third tube was sealed in the middle for the thermocouple. A cylinder of potato-tuber tissue 1.5 cm. in diameter and 2.5 cm. long was placed in the center of the cell. Platinum electrodes 1.5 cm. in diameter, connected to platinum rods passing through rubber stoppers, were placed in the glass tube against the ends of the potato cylinder. The vent tubes were necessary to permit the escape of air and thus insure good con-

¹ Condensed from a thesis presented by Catherine Personius to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1937.

tact between the platinum electrodes and the ends of the potato cylinder. A copper-constantan thermocouple was inserted into the potato cylinder through the middle opening. The other end of the thermocouple was maintained in gently boiling water in a thermos bottle. Boiling was achieved by means of a wire immersed in the water and heated by the passage of an electric current. The conductivity cell is illustrated (Fig. 1). The voltage of the thermocouple was determined using a Leeds and Northrup type K potentiometer. The resistance of the potato cylinder was measured by a Wheatstone bridge using the current from a 1,000-cycle hummer. A condenser across the resistance box was necessary.

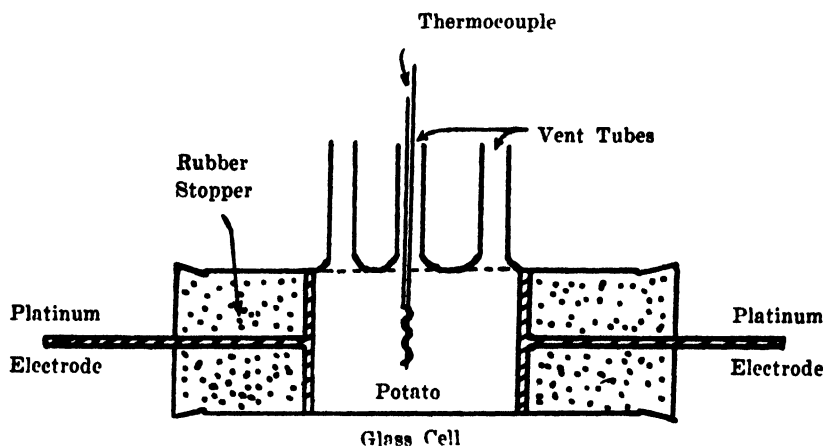


FIG. 1. Conductivity cell used for measuring electrical resistance of potato-tuber tissue.

Heating was accomplished by placing the cell in an air oven maintained at 120°C. (248°F.). Both temperature and resistance readings were taken at frequent intervals. The data are reported in terms of the total resistance in ohms offered by the cylinders of potato-tuber tissue 1.5 cm. in diameter and 2.5 cm. long.

RESISTANCE-TEMPERATURE CURVES OBTAINED ON HEATING SECTIONS FROM MEALY AND SOGGY POTATOES

Samples of potatoes from lots known to give mealy and from lots known to give soggy cooked products were tested by the procedure just described. Typical curves obtained by plotting the logarithm of resistance of the sample against internal temperature of the sample are given (Figs. 2 and 3). Logarithms were used to compress the scale of resistance values. It will be seen that both types of potatoes gave practically identical curves. The resistance decreased

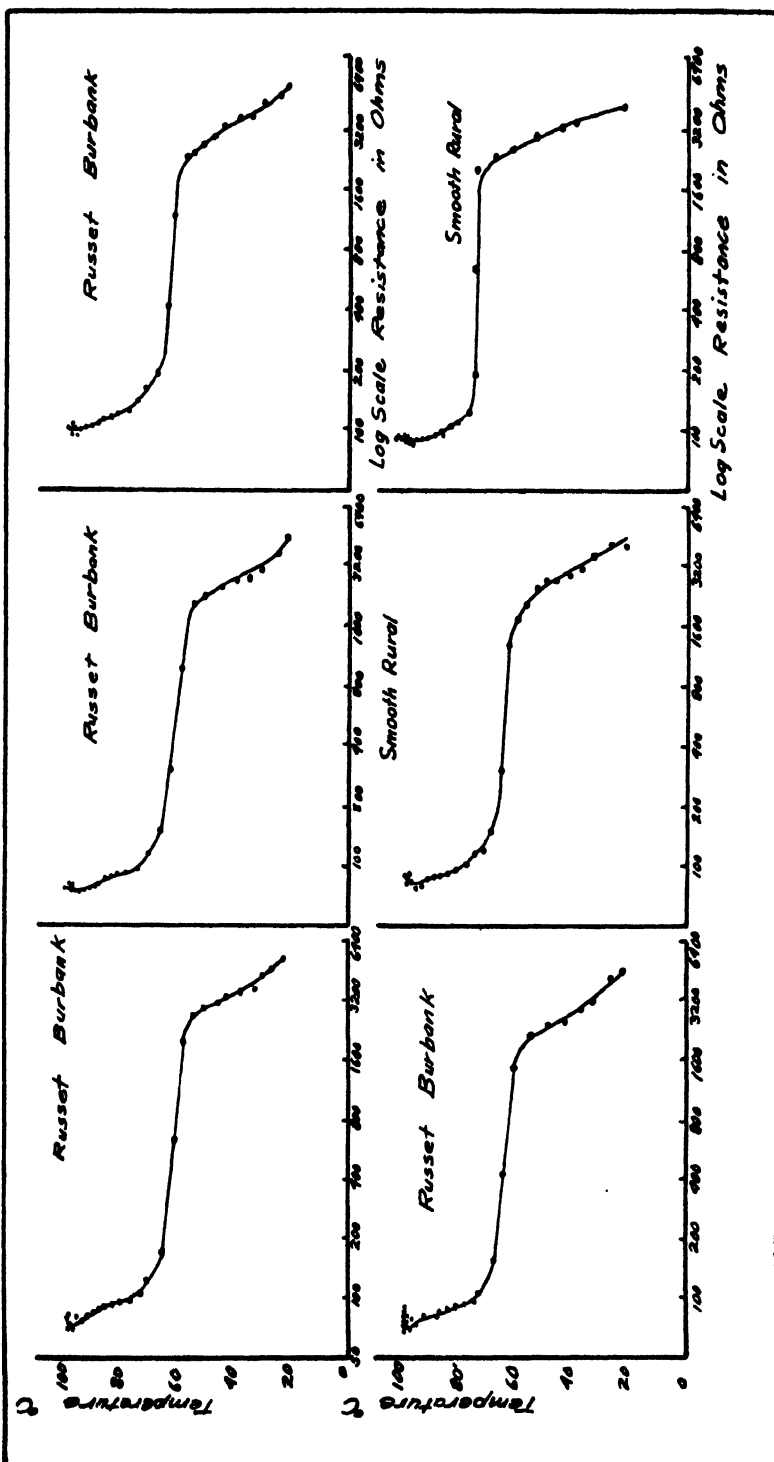


FIG. 2. Changes in resistance during heating of mealy potato-tuber tissue.

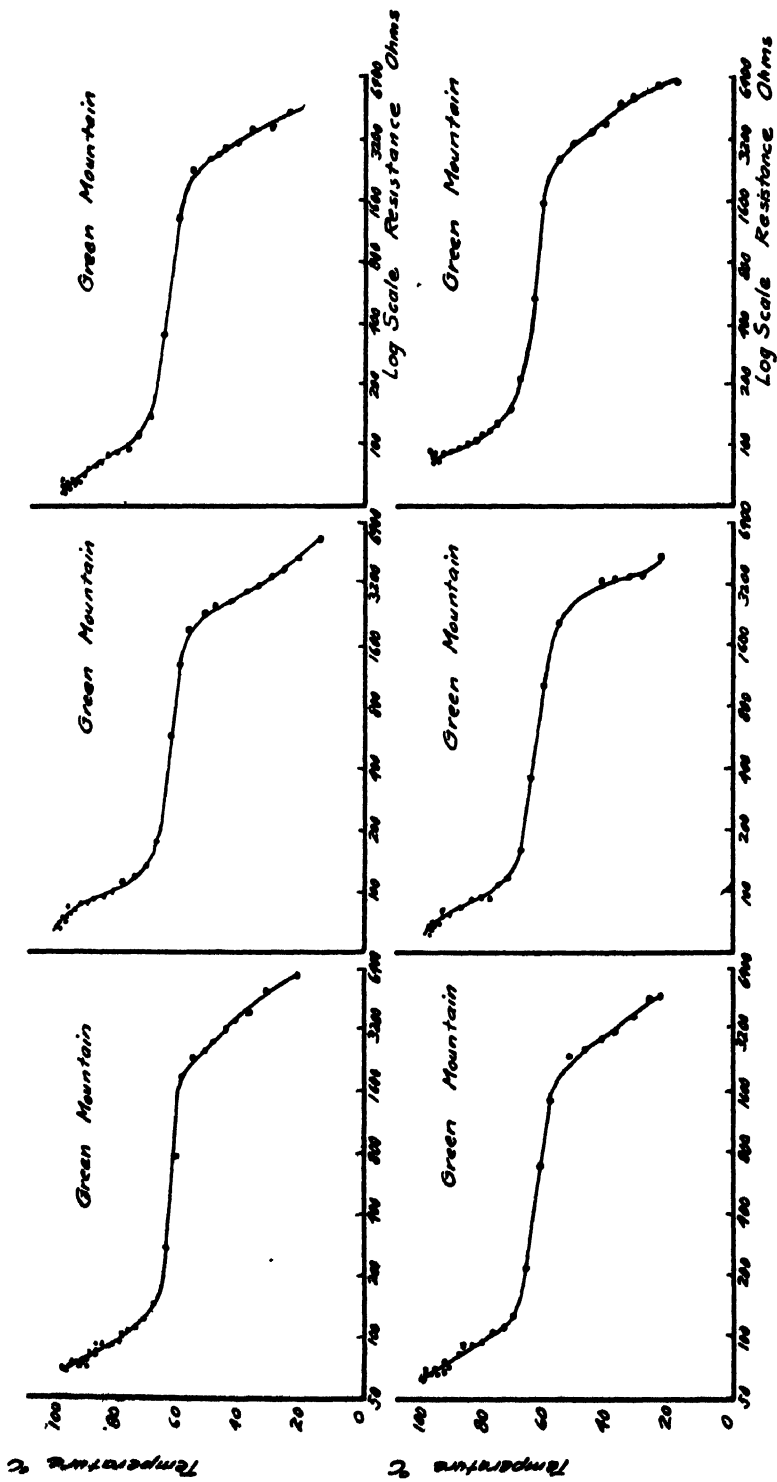


Fig. 2. Changes in resistance of vacuum tubes as a function of temperature.

slowly during the first eight or 10 minutes of heating while the internal temperature of the sample was increasing to about 60°C. (140°F.). In the next two or three minutes, while the temperature was increasing about 10 degrees, the resistance decreased tremendously, from approximately 2,000 ohms to about 200 ohms. During the remaining 15 or 20 minutes of heating the temperature increased to approximately 100°C. (212°F.) and the resistance again decreased slowly to a value somewhere between 50 and 100 ohms.

There was some variation in the absolute values obtained for the different samples, but the curves obtained by plotting the data were very similar. In the samples taken from mealy potatoes there may have been a slight tendency for the resistance values to increase somewhat during the last few minutes of the heating period. This tendency was never marked, however, and was not observed with all samples. In at least one case, a sample which was judged to be soggy showed this same characteristic.

EFFECT OF STARCH SWELLING ON RESISTANCE

The abrupt decrease in resistance which occurred regularly in the potato cylinders at a temperature between 60 and 70°C. (140 and 158°F.) indicated that the major change in permeability was taking place in the tissue at this temperature. This temperature range is the same as that at which most of the starch of the potato swells or becomes gelatinized. Swelling of the starch would be expected to cause an increase in the resistance of the sample rather than a decrease.

The temperature-resistance relationships of solutions with and without suspended starch were studied in order to eliminate the masking effect of changes in the tissue permeability. Potato juice was prepared by peeling tubers, putting them through a medium-fine grinder, and straining the pulp through cheesecloth. The resulting solution was comparatively free of starch. After standing a few minutes most of the remaining starch settled and the liquid was decanted or the juice was further freed of starch by centrifuging for 10 minutes. These juices became turbid on heating owing to the formation of a grayish flocculent precipitate which probably was largely coagulated protein. Some of the centrifuged potato juice was boiled and filtered to remove this heat-coagulable material. A concentration of 16 per cent starch was selected as being comparable to the concentration of starch in potato tissue. Commercial potato starch was used.

It will be seen (Fig. 4) that the solutions tested alone showed a regular decrease in resistance with increasing temperature. When the temperature of the starch suspensions was increased, a regular de-

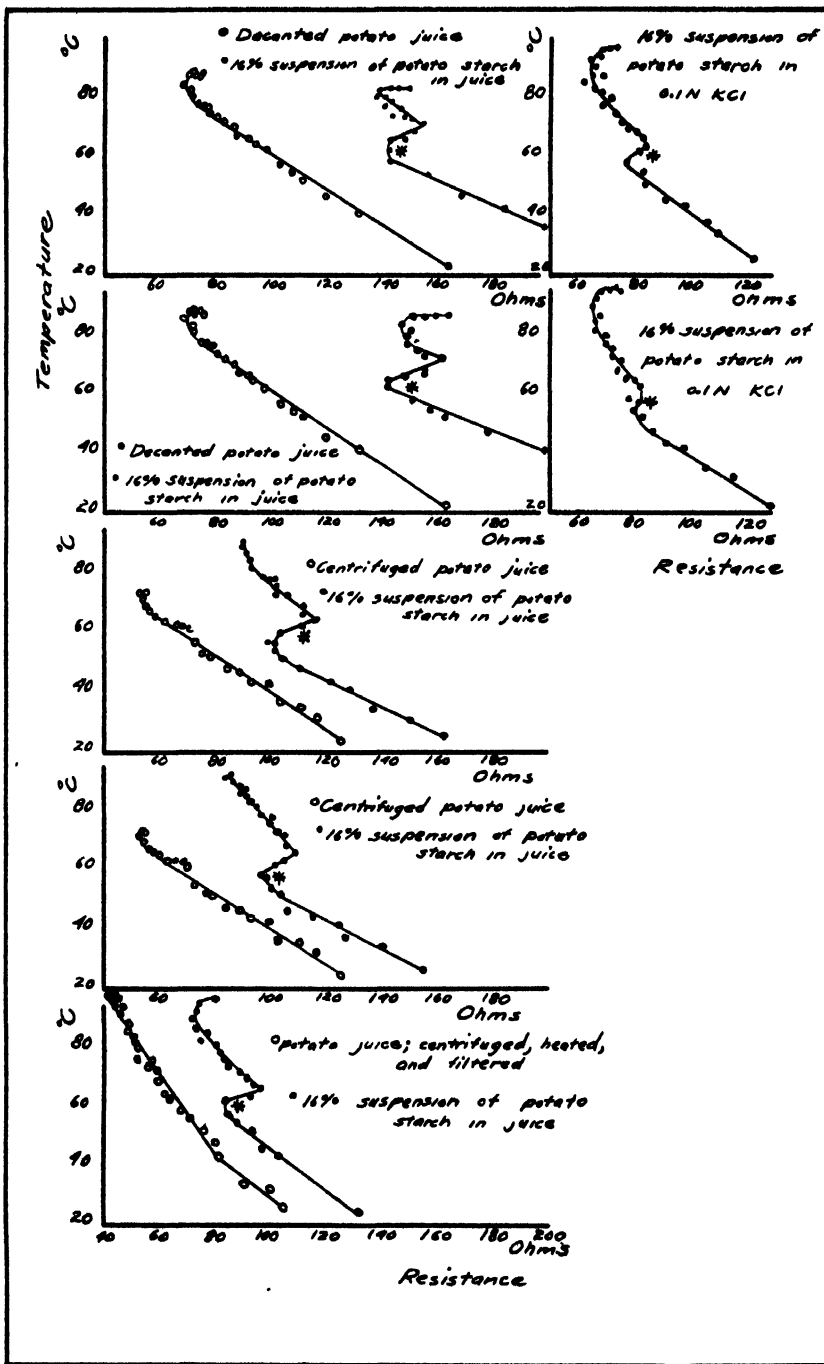


FIG. 4. Effect of starch swelling on resistance as shown by heating potato juice and KCl solutions with and without 16 per cent of potato starch in suspension.

crease in resistance occurred until a temperature somewhat above 60°C. was reached, then a small increase in resistance occurred which was again followed by a period in which the resistance decreased regularly with increasing temperature. The temperature at which the resistance began to increase coincided with the observed swelling of the starch. Experiments in which starch was suspended in 0.1 N KCl show that the increase in resistance which occurred was the result of swelling of the starch and was not due to any special property of the potato juice.

With most of the solutions a definite increase in resistance occurred during the last few minutes of heating. At this time air bubbles were beginning to form throughout the cell. The cell contents were viscous, even jelly-like, and did not allow the bubbles to escape. These undoubtedly account for the increase in resistance observed near 100°C. The 16-per cent starch suspensions in the various potato juices were found to have a resistance of about 110 ohms at 60°C. This value is similar to the resistance offered by potato tissue after it has undergone the marked decrease in resistance owing to heating.

RESISTANCE-TEMPERATURE CURVES OBTAINED BY HEATING POTATO TISSUE BY INTERNAL ELECTRICAL RESISTANCE

In the oven, heat penetrates from the outside of the resistance cell toward the center. The thermocouple indicates the temperature of the center of the section, which is perhaps lower than the average temperature of the section as a whole. For this reason heating by passing the current from the 110-volt, 60-cycle lighting circuit through the section by means of the same electrodes which were used for measuring the resistance was tried. It was thought that this method would generate heat uniformly in the potato section. If an examination of the tissue after such heating was sufficient indication, the section was not heated uniformly. This method of heating did alter the shape of the resistance-temperature curves (Fig. 5). Passage of the 110-volt current through the section markedly decreased the resistance at temperatures considerably below 60°C., the temperature at which the resistance was decreased by heat as ordinarily applied. The marked difference is at once apparent (Figs. 2 and 3 compared with Fig. 5).

PERMEABILITY AS INFLUENCED BY PASSAGE OF ELECTRIC CURRENT

The effect of passage of the current from the 110-volt alternating circuit on resistance of the tubers was investigated in some detail.

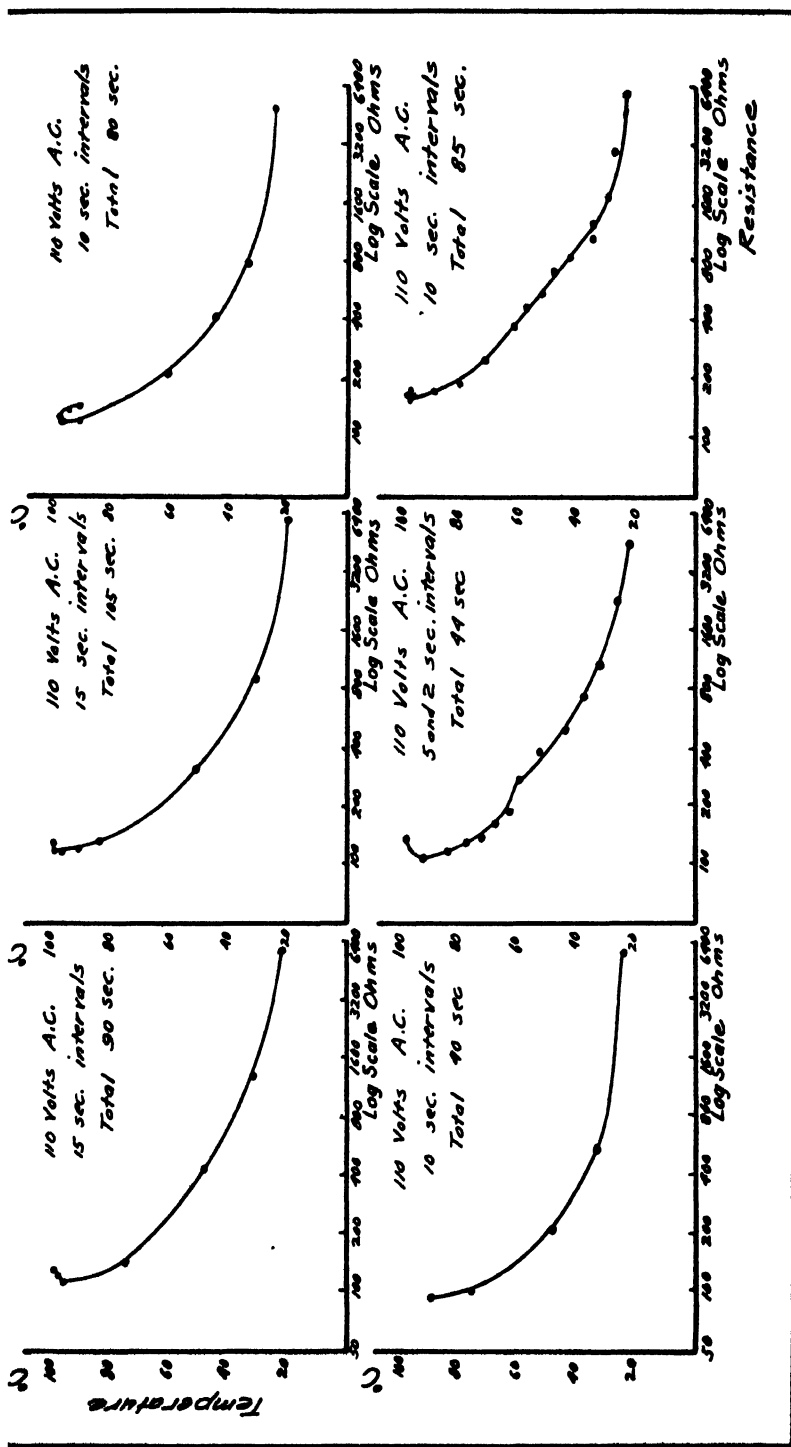


Fig. 5. Changes in resistance during heating of potato tuber tissue when heating was produced by the passage of an alternating current.

Passage of the current increased the temperature of the section, the increase being greater the longer the current was allowed to flow. Experiments were made to see if the resistance of the section could be reduced to a low value without appreciably increasing the temperature of the section. The current was allowed to flow for various short intervals of time. The section was then cooled and the current again allowed to flow. The resistance of the section could be reduced to a value approaching that of a cooked potato without the temperature of the section rising above 25 to 30°C. (77 to 86°F.). Results obtained in several experiments of this type are presented graphically (Fig. 6). The first few times the current was applied it had a greater effect on the resistance than did further applications. This was to be expected since the resistance must approach a limiting value when the material becomes freely permeable. The total decrease in resistance of the section was approximately the same for a given total interval of time irrespective of length of the individual time increments going to make up the total (Fig. 7).

After the resistance had been reduced to that characteristic of a cooked potato by the passage of an electric current without appreciable temperature increase, the tensile strength of the tuber tissue was still typical of raw potato. This is evidence that the increase in permeability and the decrease in adhesion of the cells are independent processes. After the resistance of tuber sections had been reduced by the passage of current, the surfaces of freshly cut sections quickly turned dark when exposed to air.

The permeability of turnip, carrot, and apple tissue was increased also by the passage of the alternating current.

RESISTANCE CHANGE OWING TO STARCH SWELLING IN TUBER TISSUE

A slight but definite increase in the resistance of starch suspensions at the temperature at which the starch swells is noticeable (Fig. 4). No such effect was detected in potato tissue (Figs. 2 and 3) because the effect of the starch swelling in increasing resistance was masked by the increased permeability of the tuber tissue as a result of heating. The cells may be made freely permeable, without increasing the temperature appreciably and without starch swelling, by the passage of the electric current. It was also found that holding potatoes at 47 to 48°C. (116.6 to 118.4°F.) for about 27 hours reduced their resistance to about the value for cooked potato tissue, without causing the starch to swell appreciably.

The permeability of potato tissue was increased at low temperatures to values corresponding to those for heated tissue either by

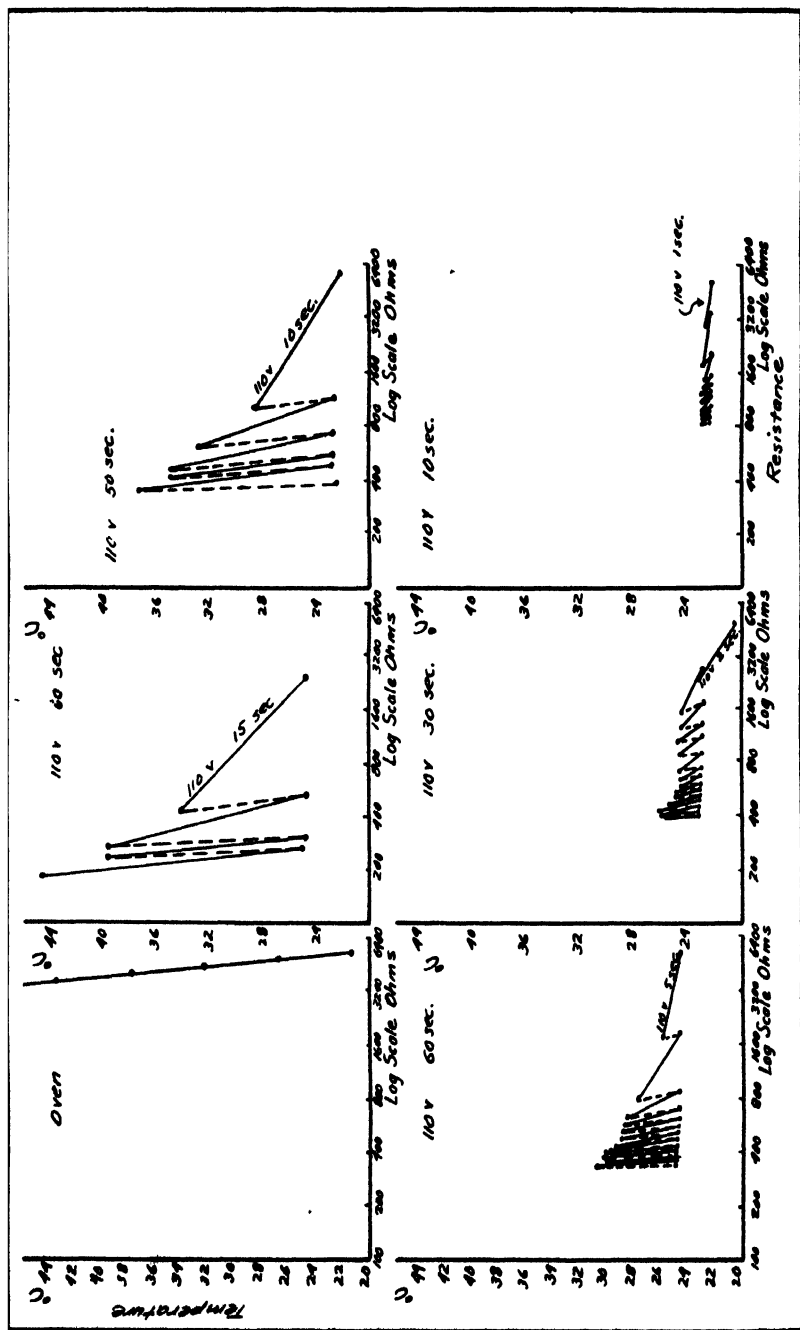


FIG. 6. Changes in resistance of potato-tuber tissue produced by passage of a 110-volt, 60-cycle current for various intervals of time; the tissue was cooled to its original temperature between intervals of passage of the current.

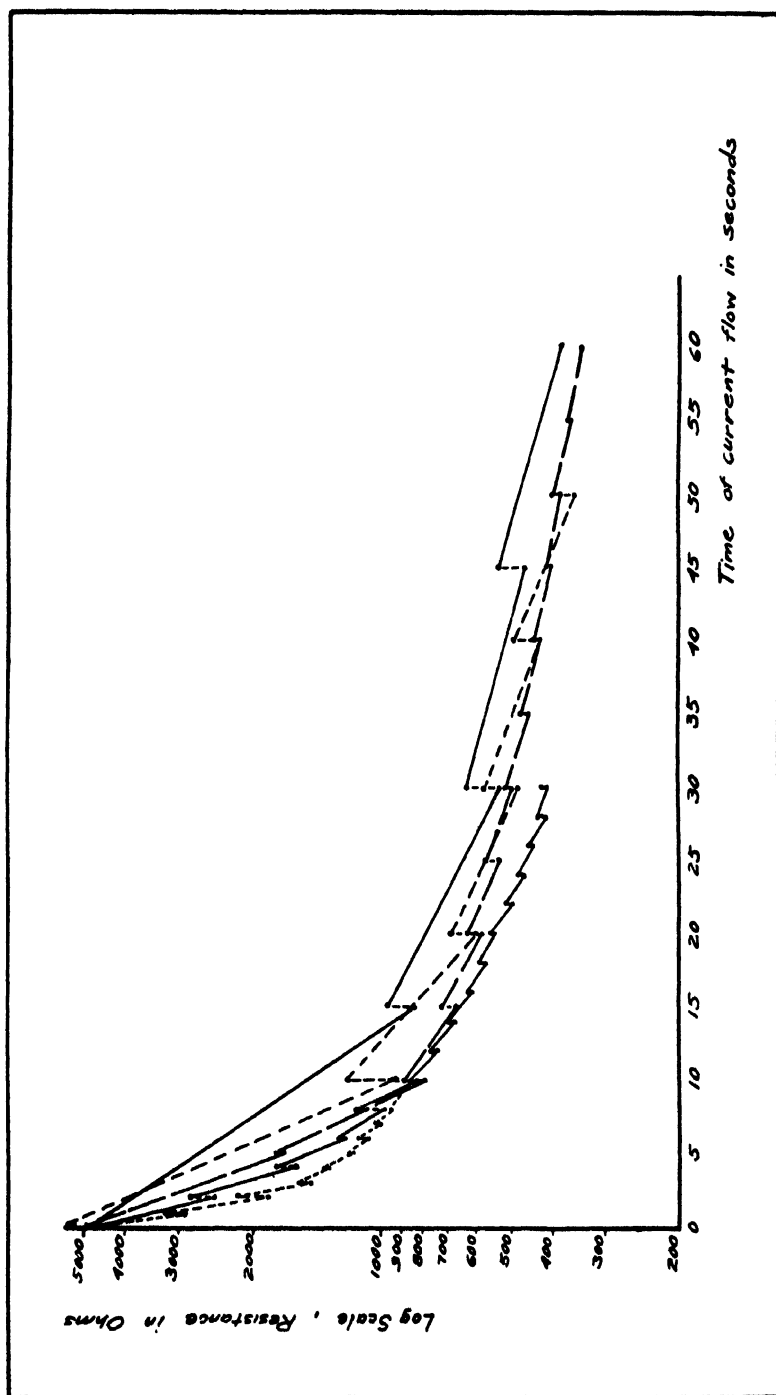


Fig. 7. Effect of length of exposure interval on the total decrease in resistance produced in a given time of flow of a 110-volt, 60-cycle current.

means of electric current or by holding at 48°C. Then sections were placed in the conductivity cell and the resistance-temperature changes during heating determined (Fig. 8). These curves, which are similar to those of Fig. 4, show clearly an increase in tissue resistance at the swelling temperature of the starch when present in the unruptured tissue cells.

DISCUSSION

Changes in electrical resistance were used to indicate the effect of heat on the permeability of potato tissue. Although long holding at 48°C.(118.4°F.) reduced the resistance to values characteristic of cooked tissue, when the tissue was heated more rapidly at a rate comparable to that used in cooking, the most marked effect of temperature on permeability occurred at slightly above 60°C.(140°F.). The resistance of the sections fell from 4,000 to 5,000 ohms at 20°C. (68°F.) to less than 100 ohms at 98°C.(208.4°F.). A slight increase in resistance owing to the swelling of the starch was noted. This effect occurred at a little above 60°C. No difference in the effect of temperature on change in permeability of mealy and soggy potatoes was noted.

Little or no formation of intercellular air spaces took place in the potato tissue as a result of heating. This was indicated by failure of the resistance to increase appreciably at the end of the cooking period.

The passage of a 110-volt, 60-cycle alternating current increased the tissue permeability markedly. It was found possible to increase the permeability to that typical of cooked potato tissue without increasing the recorded temperature above 25 to 30°C.(77 to 86°F.), and undoubtedly the permeability could have been increased at still lower temperatures. The mechanism by which the passage of current increases permeability is most easily explained by assuming localized heating of parts of the cells which go to make up the tissue as a whole. The thermocouple measures the average temperature of a number of cells at the center of the cylinder. The portions of the cell offering the greatest resistance to passage of the current would be heated the most and would be the portions having semi-permeability or selective permeability. This localized heating would destroy the semi-permeable property of the tissue.

This method of applying heat locally to specific parts of the tissue opens up many possibilities for investigation. It enables one to produce profound localized alterations in the tissue without altering its main structural elements. The passage of the electric current was found to decrease the resistance of white turnip, carrot, and apple tissue and the effect is probably general for tissue of this type.

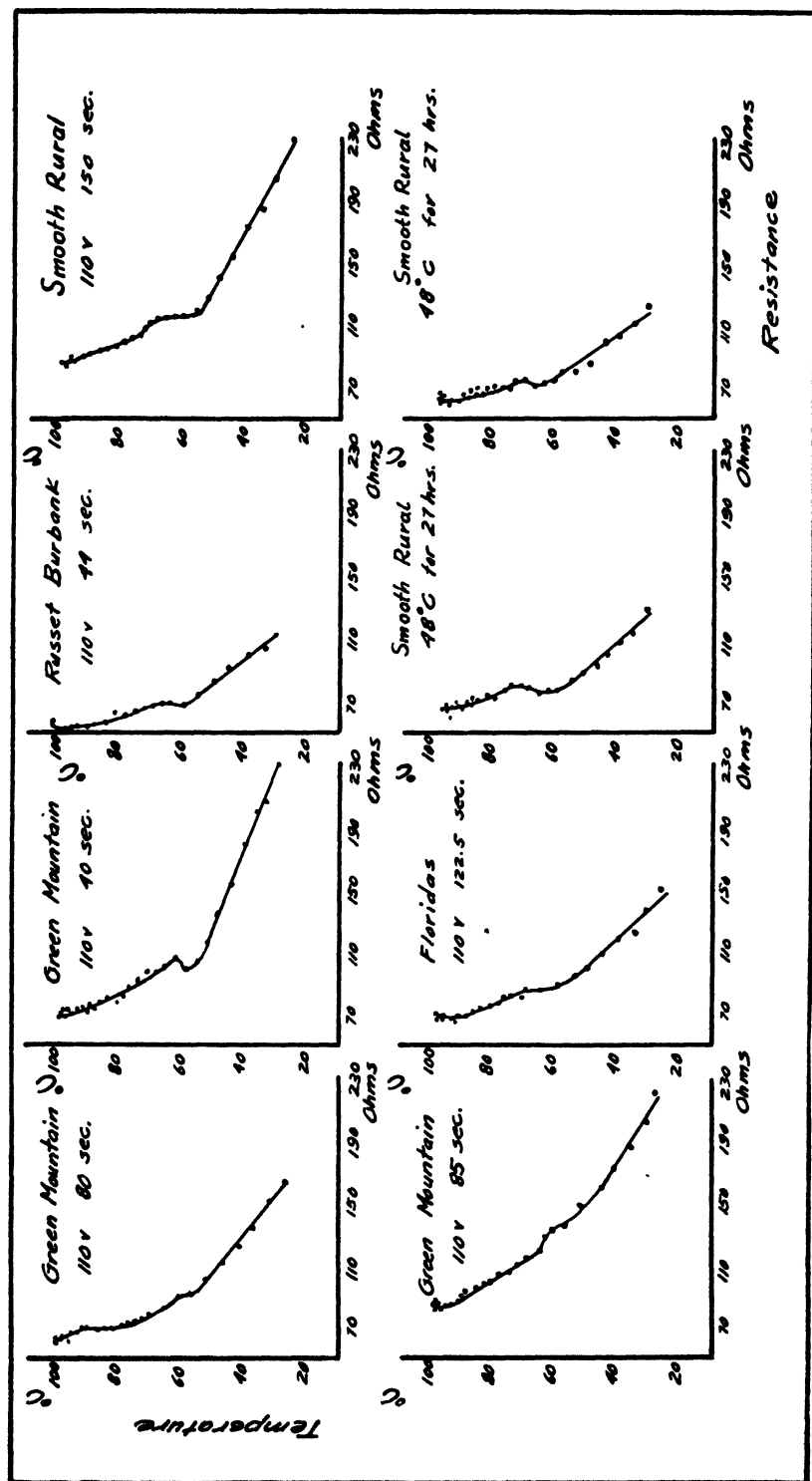


FIG. 8. Effect of starch swelling on resistance of potato tissue as shown by heating samples, the resistance of which had been lowered previously without starch gelatinization by the passage of an electric current or by holding at 48°C. (118.4°F.).

CONCLUSIONS

1. The change in electrical resistance of potato-tuber tissue was found to be a satisfactory method for studying changes in permeability.

2. In normal cooking, potato tissue permeability was increased most sharply at a temperature slightly above 60°C.(140°F.).

3. Potato tissue was rendered freely permeable by the passage, for a few short intervals, of a 110-volt alternating current without increasing the measurable temperature of the tissue above 30°C.(86°F.).

4. Holding potato tissue at 48°C.(118.4°F.) for 27 hours destroyed the semi-permeable properties of the potato tissue.

5. No significant difference in the effect of heat or electric current on the permeability characteristics of soggy and mealy potatoes was noted.

6. The effect of electric current in increasing permeability may be assumed to be due to production, by the semi-permeable membranes, of localized regions of high resistance, thus producing a localized heating to a temperature sufficiently high to destroy the semi-permeable properties of the membrane.

7. The marked increase in permeability owing to the effect of the electric current is accompanied by greater enzyme activity as shown by darkening of the tissue when a cut surface is exposed to air.

8. Potato tissue may be rendered freely permeable without altering the adhesion of the cells or causing a gelatinization or swelling of the starch.

9. Heat gelatinization or swelling of starch suspended in a salt solution is accompanied by a slight increase in electrical resistance.

10. Little or no formation of intercellular air spaces in potato tissue occurs during cooking.

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DEGREE OF PIGMENTATION AND ITS PROBABLE RELATIONSHIP TO THE DIASTATIC ACTIVITY OF HONEY

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Although conflicting statements have been made in the past as to the nature and variety of enzymes which honey contains, it is now generally accepted that the number is limited to three: an invertase, diastase, and catalase. The reason for the presence of the first of this group, which is secreted by the bee, is obvious since honey is essentially a syrup of invert sugar. Less understood are the origins and purposes of the others. The presence of diastase has been made, on the one hand, the point of departure in the development of methods for distinguishing between either genuine and sophisticated honeys or heated and unheated ones and, on the other, the formulation of a legal definition of this food by the authorities of at least one foreign country.

For literature reviews on the subject of honey enzymes the reader is referred to papers by Moureau (1911) for the period 1873 to 1910 and by Gothe (1914) whose more elaborate survey concludes with his contribution to the measurement of diastatic activity (1914a). His mode of procedure has been modified by Lothrop and Paine (1931) who saw the desirability of controlling the pH of the reaction mixtures that the full hydrolytic power of this enzyme might be utilized. Schuette and Pauly (1933) pointed out, in a sense, that any data expressing diastatic activity can only have a real quantitative significance when a standardized substrate shall have been designated. The latter communications are pertinent to this one in that they predict the analytical technic which was used in seeking an answer to the question whether increasing intensity of color in a series of differently pigmented honeys might not be reflected in a rise in diastatic activity.

EXPERIMENTAL WORK

Among the 20 samples used in this investigation were representatives of the whole gamut of color classification which is used in the merchandising of honey, from the so-called water-white and white through light amber and amber to dark. They were obtained from growers in six states and were received for the most part in the comb with the honey sealed in the cell by the bee and, therefore, in no way affected by any external heat treatment. The exceptions were

several dark honeys from the South which were received in liquid form but with their diastatic activity apparently little, if any, impaired by any heating to which they might have been subjected in the process of extracting them from the comb. From several apiaries in Illinois were obtained honeys whose respective floral sources were two varieties of clover, sweet and an unnamed one, and mixed fall flowers. The Wisconsin group contained the same types but with the addition of buckwheat. From California was obtained a sample of orange-blossom honey; from Colorado, alfalfa and alfalfa-clover mixtures; from New York, several buckwheat honeys as well as two varieties of clover. Gallberry, Mexican clover, tupelo gum, and thistle honeys were used as typical of those produced in Georgia.

Described elsewhere by Schuette and Pauly (1933) is the experimental procedure used throughout this study, the composition of the buffered substrate mixture, and the preparation of the 10 sample

TABLE 1
Color Grade of Honey in Relation to Diastatic Activity

Color grade	Number of honeys examined	Pfund scale reading ¹	Diastatic activity
Water-white.....	3	0.5	14
White.....	5	2.5	16
Light amber.....	7	5.4	28
Amber.....	2	9.4	57
Dark.....	3	12.7	122

¹ The color number of each specimen was determined with the aid of a U. S. Standard honey grader whose scale (Pfund) is so calibrated that an increase in numerical values represents a corresponding intensity in pigmentation.

tubes which are necessary in carrying out this determination. As before, the tube showing a true purple color immediately preceding the first one in the series in which a true blue color was developed upon the addition of one drop of iodine, was used as the basis for calculating the diastase value.

Data for each of the 20 honeys which were examined have not been made a part of the record because degree of pigmentation rather than floral source was primarily the point of departure herein involved. To that end, average color values and diastatic activities only are presented (Table 1).

DISCUSSION

If the data (Table 1) are plotted, a smooth curve will be obtained which, in spite of its regularity, has probably no mathematical significance. Qualitatively, however, one interpretation is obvious; the darker the honey the greater is its diastatic activity. The

differences in this form of biological activity, although distinct, do not increase rapidly in the three lower color grades but from this point on they move apart with large strides. When expressed in terms of floral types, the data reveal that buckwheat honey and perhaps, too, that produced from flowers maturing in the fall and those of southern origin, such as gallberry and tupelo gum, are far superior in this respect to the more delicately flavored products of the various varieties of clover, alfalfa, or orange blossoms.

Reference has been made above to the fact that the source of diastase in honey is little understood. Several investigators, by approaching the problem from different angles, have attempted to account for its presence in this food. Küstenmacher (1908), unable to detect any degradation products in the reaction mixture when pollen in aqueous suspension was allowed to react, first for one hour and then longer, upon a one-per cent solution of starch, concluded that its origins lie in the bee itself. Gothe (1914), on comparing the relative potencies of extracts of pollen with those prepared from desiccated bees, averred that a honey is indebted to both bees and plant for whatever of this enzyme it may contain. But Vansell and Freeborn (1929), on the basis of an observed rough proportionality between diastatic activity and the number of pollen grains, credited the latter with being the source of this ferment. With this conclusion Lothrop and Paine (1931), who determined the diastatic activities of several representative floral types, are in agreement. Our own measurements in this field do not permit us to reach the same conclusion.

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MINERAL CONSTITUENTS OF HONEY

III. SULFUR AND CHLORINE

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In so far as a careful search of the literature reveals, it appears that priority of communication on sulfur and chlorine as constituents of honey might properly be given to Lenz (1884) who, some 53 years ago, reported that he had identified these elements in eight samples of widely separated geographical origins. Not until almost a decade thereafter were pertinent quantitative data available when Villaret (1893) found that the sulfur content of the series of 48 Russian honeys which he had examined comprised from 2.46 to 6.90 per cent of the ash. The gap was filled in a sense by Van Dine and Thompson (1908) whose fairly complete analyses of the ash of single samples of Hawaiian algaroba and honey-dew honeys furnished the first clues as to the probable order of magnitude of the chloride content of this food, or 30 and 667 parts per million, respectively.

Since that time more information on this subject has been made available in the communications of a number of other investigators to whom, but for several minor exceptions, reference has already been made in the preceding report of this series. To this group should be added the names of Nottbohm and associates (1911, 1913, and 1914) whose studies in this field of honey chemistry, particularly chlorine, are significant.

Analysis of the data reported by the above group leads to the following observations: (1) in contrast to the elements of calcium, magnesium, and phosphorus, the presence of chlorine and sulfur in honey has not yet been explained beyond the fact that Hawaiian honeys are notably high in the amount of the former which they contain; (2) the present known limits of sulfur and chlorine contents are, respectively, 14 to 124 and 3 to 2,545 parts per million; (3) an almost total lack of information on this point in respect to honeys grown in the United States makes a survey thereof desirable.

The results of such a survey, made upon 23 honeys of diverse floral sources which are deemed to be representative of the bee pasture in California, Colorado, Georgia, New York, Illinois, and Wisconsin, are herein presented.

EXPERIMENTAL PROCEDURE

Determination of Chlorine: The well-known Volhard method was used for the volumetric determination of chlorine in aliquot portions of nitric acid solutions of the ash of 12.5 grams of honey which had been prepared from sample weights four times as large. Retention of chloride was assured by the addition of sodium carbonate solution to the honey before incineration at 500°C. (932°F.). Recovery experiments on three controls containing sodium chloride solution (1.56 mg. Cl equivalent), saccharose having been substituted for honey, showed a mean deviation of $\pm .93$ mg. or *ca* two per cent.

Determination of Sulfur: For the determination of sulfur, advantage was taken of the Schroeder (1933) and Sheen, Kahler, and

TABLE 1
Ash, Sulfur, and Chlorine Contents of Honey

Classification	Samples	Ash	Sulfur		Chlorine	
			Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.
Light honeys		<i>pct.</i>				
Water-white.....	3	.042	12.45	50	11.64	50
White.....	7	.064	10.53	67	8.77	54
Average.....053	11.49	58	10.20	52
Dark honeys						
Light amber.....	8	.127	6.83	80	8.76	75
Amber.....	3	.221	4.87	98	7.03	131
Dark.....	2	.102	12.26	122	13.22	133
Average.....150	7.98	100	9.67	113

Cline (1937) technique of direct titration of sulfate with standard barium chloride solution in the presence of tetrahydroxyquinone as internal indicator. Wet-oxidation of the honey with a perchloric-nitric acid mixture was used in the preparation of the ash solution in this instance. Recovery experiments, along lines indicated above, made for the purpose of demonstrating the efficiency of the oxidation procedure and the accuracy of this method, in four trials showed a mean deviation of $\pm .7$ per cent when .8 to 2 mg. of sulfur were taken.

RESULTS

Data are recorded by color groups according to position on the Pfund scale (Table 1) rather than individuals, for reasons explained in an earlier communication. To the light-honey group were assigned again the water-white and the white honeys; to the deeper-pigmented group the light amber, the amber, and the dark honeys. The effect

of this segregation is that the first group consists of honeys whose predominating floral sources are clover, alfalfa, white sage, and orange blossoms, respectively; the second consists of buckwheat, wild aster, and other late-blooming flowers and representatives of southern nectar sources, such as gallberry, tupelo gum, Mexican clover, and titi. Since this compilation does not reveal the limits within which the elements in question occur in each main division, the variations in content thereof have been made the subject of a separate one (Table 2).

DISCUSSION

With the completion of this study of the sulfur and chlorine contents of 23 honeys which had been produced exclusively in continental United States there comes now an opportunity to make com-

TABLE 2
*Variation in Sulfur and Chlorine Contents of
Light and Dark Floral Honeys*

Classification	Sulfur		Chlorine	
	Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.
Light honeys				
Minimum.....	5.77	36	4.52	23
Maximum.....	16.24	108	13.21	75
Dark honeys				
Minimum.....	2.67	56	2.26	48
Maximum.....	14.36	126	14.46	201

parison with similar data pertinent to honeys of other geographical origins (Table 3). Hitherto such a comparison has been impossible because of insufficient data. Apparently the only available source of information with respect to the sulfur content of a domestic honey (40 parts per million) is a communication by Kemmerer and Boutwell (1932) in which there has been recorded, along with 95 other foods, the analysis of a single sample. Similarly, to date the source of information on chlorine content has been the report by Lendrich and Nottbohm (1911) of the results of their analyses of German imported honeys, among which was a single sample from California which was found to contain 300 parts per million.

With their observed 36-to-126 mg. per kg. range, American honeys appear to fit rather well into the sulfur picture (Table 3). When, however, the sulfur content is expressed in its relationship to the quantity of ash which the honey contains (2.67 to 16.24 per cent) no such small quantities (.24 to .46 per cent) which constitute the present known minima were found. The order of magnitude of the

maxima, on the other hand, are substantially alike, or 16 and 13 per cent, respectively. It is to be regretted that data on more than two Hawaiian honeys are not available; then a convincing comparison might be made with those at hand. Consequently, in the light of present information and the implied limitation, it appears that the product of these islands is probably lower in sulfur content than that grown on the mainland. The single report from Australia suggests that the honeys produced there are of a lower sulfur content than those grown in the United States.

The suggestion of similarity which is noticeable in the sulfur content of United States honeys and those of other countries for which data are available, is not apparent in the case of chlorine

TABLE 3
*Classified Summary of Sulfur and Chlorine Contents of Floral Honeys
Reported During the Period 1893-1933*

Origin	Sulfur		Chlorine	
	Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.
Australia.....	20-60 ⁵ *	170-1070 ⁵
Europe.....	.46-13.02 ⁷²	18-124 ⁶	.32-4.44 ²⁵	3-442 ²⁶
Hawaii.....	.24-0.32 ²	14-30 ²	17.37 ¹	30-2545 ¹⁸
South America.....	1394-1515 ²
United States.....	40 ¹	300 ¹
West Indies.....	78-1272 ⁵
Summary.....	.24-13.02 ⁷⁴	14-124 ¹⁴	.32-17.37 ²⁶	3-2545 ⁵⁸

* Superior numbers indicate the number of samples involved in this survey

(23 to 201 mg. per kg.) except, perhaps, for honeys of European origins. On the other hand, the products of Australia, South America, the West Indies, and notably the Hawaiian Islands, separately and collectively, so extend the known upper limits of the chlorine content of this food (2,545 mg. per kg.) as to make the difference an emphatic one. The lowest value of the Hawaiian group of 18 samples is that reported by Van Dine and Thompson (1908); the next step in this series begins at 1,998 mg., the smallest quantity of chlorine which was found in 17 samples. The latter group was analyzed by Nottbohm and associates (1911 and 1914) who, aware of inevitable chlorine losses during incineration, added sodium carbonate in order to overcome this source of error. The record does not reveal that Thompson used any such precautionary measures.

Both sulfur and chlorine content rise with increasing degree of pigmentation (Table 1). The dark honeys were found to contain more of these elements than did the light honeys, the difference being more marked in chlorine than in sulfur content. These rela-

tionships are deemed to have a qualitative, but not a quantitative, significance.

SUMMARY

As a result of the analysis of 23 honeys deemed to include representatives of most of the honeys produced commercially in the United States, it appears that there apparently exists a qualitative relationship between degree of pigmentation as measured by the current practice of color-grading this food and the quantity of sulfur and chlorine which it contains. All available data on this phase of honey composition have been collected and collated. Although some measure of similarity in sulfur content exists between the honeys in question and those produced in the foreign countries included in this survey, such has not been found to hold true for chlorine. Honeys of European origins probably do not contain notably greater amounts of this element than do those grown in continental United States, but no such large chlorine content has been found as that reported by others for the honeys characteristic of the product of the Hawaiian Islands nor yet those from the West Indies, South America, and Australia. There is need, however, for more data on the honeys from the latter group of geographical units, except perhaps the Hawaiian Islands, because the number of samples here involved is small.

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NUTRITIVE VALUE FOR GROWTH OF SOME PROTEINS OF FISHERY PRODUCTS

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The nutritive value has been determined for many proteins of other foods, but only a limited amount of information is available concerning the comparative value of fish protein. Drummond (1918) investigated the value of the muscle of cod, herring, and salmon. He found no differences in the growth-promoting value of these fish proteins and of beef, but casein seemed to be distinctly inferior when fed at a level of six per cent in the diet. These results were confirmed by Suzuki and associates (1919). Kik (1927) investigated the quality of the proteins of haddock and herring and found that both produced satisfactory growth but herring seemed to be somewhat superior to haddock. Holmes (1918) found that Boston mackerel was digested to the extent of 93.1 per cent by human subjects. Jones (1926) found

the ratio values of $\frac{\text{gain in weight}}{\text{gram of protein}}$ for shrimp, clam, and oyster to be 2.15, 2.05, and 1.27 respectively.

Because fish flesh is a common constituent of the human diet, and since it is eaten primarily as a source of protein, this investigation was undertaken to determine the comparative nutritive values, for growth, of the edible portion of a number of common fish and shellfish.

Albino rats bred from stock animals in the Nutrition Laboratory of the U. S. Bureau of Fisheries at College Park, Maryland, were selected at an initial live weight of about 50 grams. They were caged individually in wire-bottomed cages, weighed twice a week, and accurate food intake records were kept. The experimental period was 10 weeks.

The diets were composed of the following constituents: extracted flesh from the edible portion of fish and shellfish was used to supply the desired level of protein; butter fat, 8; Osborne and Mendell salt

¹Part of the data included in this paper was incorporated in a thesis submitted by William B. Lanham, Jr. to the graduate school of the University of Maryland in partial fulfillment of the requirements for the degree of Master of Science, June, 1937. The authors wish to acknowledge the assistance and advice of Dr. F. P. Griffiths in planning the preliminary experiments in this series.

mixture plus a trace of copper and zinc, 4; a commercial vitamin concentrate from milk (Dry Milk Company, Inc.), 4; alcoholic extract of wheat embryo, 10; cod liver oil, 2; and dextrin to make 100 parts by weight.

The fish and beef proteins were prepared by covering the ground flesh with acetone and permitting it to stand for about 10 hours. This extraction was repeated three times, after which the resulting residue was reground, and then continuously extracted for 14 hours with fresh acetone in the extractor designed by Lemon, Griffiths, and Stansby (1936). The residue was finally dried on a steam bath in the open air to remove the acetone. After grinding in a Wiley mill,

TABLE 1
Analyses of Protein Concentrates Used in Experiments

Source of protein	Weight			
	Moisture	Ash	Ether extract	Protein (NX 6.25)
	<i>pct.</i>	<i>pct.</i>	<i>pct.</i>	<i>pct.</i>
Cod.....	6.13	2.70	0.08	93.28
Croaker.....	5.59	2.78	0.21	94.03
Shad.....	5.92	3.30	4.55	85.85
Boston mackerel.....	6.56	3.94	1.94	90.32
Oyster.....	8.77	6.22	0.58	62.27
Red snapper.....	8.73	2.68	0.05	93.33
Silver salmon.....	8.05	2.67	0.16	88.57
Pilchard.....	7.22	4.35	0.09	86.81
Shrimp.....	7.71	4.52	0.30	91.00
Haddock, extracted.....	10.74	3.13	0.18	88.50
Haddock, dried.....	5.92	4.89	1.29	89.31
Casein.....	8.50	2.36	0.05	83.00
Beef.....	6.56	2.25	1.46	91.28

the final product was a light, dry meal which could be stored indefinitely in sealed Mason jars. Domestic, acid-precipitated casein was used. Ground round steak was chosen as a typical cut of beef. Typical analyses of the prepared fish proteins with those of beef and casein are presented (Table 1).

The protein in the remaining constituents of the diet were alcoholic extract of wheat embryo, 1.55; cod liver oil, .03; dextrin, .21; butterfat, none; milk vitamin concentrate, 9.89 per cent by weight. All nitrogen determinations were made by the Kjeldahl method modified according to Gerritz and St. John (1935) and Markley and Hann (1925).

PRELIMINARY EXPERIMENTS

Haddock protein was fed at levels of 6, 9, 15, 18, and 21 per cent by weight in the diet in order to determine the level necessary to

promote maximum growth. Eighteen per cent casein in a similar diet served as a control. Less than 18 per cent protein in the diet permitted only subnormal growth, so the level of protein was apparently the limiting factor (Table 2).

TABLE 2
*Average Intake of Food and Ratio of Growth to Protein Intake of Rats
on Extracted Haddock and Casein Diets*

Diet	Number of rats	Food intake	Protein intake	Gain in weight	Gain in wt. per gm. protein
6% Haddock.....	6	598	37.6	61.5	1.64
9% Haddock.....	6	647	61.9	77.4	1.25
15% Haddock.....	6	637	101.0	89.3	0.88
18% Haddock.....	5	598	112.2	104.0	0.93
21% Haddock.....	6	640	139.3	90.8	0.65
18% Casein.....	6	642	104.8	107.9	1.03

In order to determine whether the acetone treatment had in any way decreased the nutritive value of the protein, two groups of rats were fed haddock protein at a nine-per cent level. One group received acetone-dehydrated haddock and the other, haddock which had been dried on a steam bath. There was no destruction of nutri-

TABLE 3
Comparison of Acetone-Dehydrated and Dried-Haddock Proteins

Diet	Number of rats	Gain in wt per gm. of protein	Standard error of ratio
9% Haddock, extracted.....	7	1.41	.167
9% Haddock, dried.....	7	1.78	.103

tive value (Table 3) since the ratio gain in weight per gram of protein for the two groups was not significantly different. Furthermore, the determination of the apparent digestibility of three of these proteins showed very small differences (Table 4).

TABLE 4
Apparent Digestibility of Proteins

Diet	Number of rats	Average digestibility coefficient	Mean of duplicates
18% Haddock, extracted.....	4	91.7	91.87 \pm 0.28
18% Haddock, extracted.....	4	92.0	
18% Haddock, dried.....	3	90.5	
18% Haddock, dried.....	4	90.4	90.45 \pm 0.36
18% Boston mackerel.....	5	89.2	
18% Boston mackerel.....	5	88.0	88.60 \pm 0.54

COMPARISON OF NINE FISH PROTEINS IN GROWTH STUDIES

The preliminary experiments indicated that protein was the limiting factor in the diets containing a 15-per cent level or less by weight; therefore, a nine-per cent level in the diet should be satisfactory for comparing growth value of proteins of the various fishery products. Male albino rats were weaned at a live weight of 49 to 54 grams and fed the fish protein diets *ad libitum* for a 10-week period. Owing to a lack of time and equipment, not more than four groups of animals were allotted at a time. A control group was fed beef protein. The results of the 10-week feeding tests show that the protein intakes were not equal for all groups. However, the gains in weight were adjusted for the differences in protein intake (Table 5) by employing

TABLE 5
Ratio of Gain in Weight per Gram of Protein and Adjusted Gains in Weight for Different Protein Intakes

Protein	Number of rats	Average gain in weight	Average protein intake	Gain in wt. per gm. protein	Standard error	Adjusted gains in weight
		<i>gm.</i>	<i>gm.</i>			
Shad.....	16	117.8	59.43	1.96	.059	100.4
Cod.....	16	105.9	52.10	1.96	.072	102.4
Croaker.....	16	121.8	59.52	2.03	.052	104.1
Boston mackerel.....	12	122.7	54.62	2.23	.064	119.0
Silver salmon.....	11	104.4	49.40	2.14	.034	107.2
Pilehard.....	11	96.9	46.75	2.03	.054	115.6
Red-snapper.....	10	88.4	46.72	1.88	.027	115.6
Oyster.....	11	150.1	60.25	2.47	.029	130.3
Shrimp.....	23	96.0	47.40	2.00	.075	112.8
Beef.....	12	95.2	58.00	1.64	.048	81.9

¹ Differences necessary for significance between any two mean gains in weight after adjustment are 8.9 gm. for odds of 19 to one and 11.8 gm. for odds of 99 to one.

Fisher's method of covariant analysis, according to Titus and Harshaw (1935). The ratio of gain in weight per gram of protein ingested tends to increase with larger protein intakes within any group. On a low intake the fraction used for maintenance is of relatively greater importance in calculating the ratio value, since less is used for energy than in the higher levels of intake.

These experiments show that the proteins of the fishery products which were tested are of excellent quality, since they compare very favorably with the protein of round steak and casein. As might be expected, they were not all of equal growth-promoting value. Oyster protein was markedly superior to the others, and apparently the fish containing 10 per cent or more of fat yielded better proteins than those containing less.

SUMMARY

The proteins from the edible portion of nine fishery products were compared at a level of nine per cent by weight in the basal diet, since it was found that the protein source at this level was the limiting factor for growth. Beef was fed as a control in a similar diet. There were no practical differences in apparent digestibility.

The proteins fell approximately into the following groups according to relative growth-promoting value:

100	90	80	63
Oyster	Pilchard	Shad	Beef
	Red snapper	Cod	
	Shrimp	Croaker	
	Boston mackerel	Silver salmon	

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ACTION OF GUM GUAIAECUM UPON THE ANIMAL ORGANISM¹

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The resinous gum of the tropical guaiacum tree has been known to medicine for centuries and has been used extensively in the treatment of various ailments, especially syphilis—Freind (1927) and Wootton (1910)—for which it was considered a specific. Quincy (1749) describes its use in gout, dropsies, "cutaneous foulnesses," catarrhs, ulcerations, gleet, and gonorrhea. The gum was believed to be a "sweetener and cleanser of the blood," a diaphoretic, and was used for "cleansing the joints" and "warming and strengthening the nerves," according to Quincy.

The claims made for the beneficial actions of guaiacum were of course exaggerated, and the substance has no place in the armamentarium of modern medicine though even yet Webster's dictionary (1932) defines the resin as being "used medicinally as a remedy for gout, rheumatism, and skin diseases."

Recent experimental work has shown, however, that guaiacum may come to occupy a useful place of importance economically and to public health. Grettie (1933) has shown that the addition of very small quantities of the gum to lard (.05 gm. in 100 gm. of lard) greatly retards the development of oxidative rancidity. Evans (1932), in a review on vitamin E, emphasizes the destructive action of rancidity in fats upon vitamin E, stating that "so easily is vitamin E destroyed by slight changes in the rancidity of the fats in which it is carried in solution that any analysis of vitamin E without simultaneous analysis for anti-oxygenic activity renders vitamin E analysis worthless." These findings led Johnson, Carlson, and Bergstrom (1938) to test whether the addition of guaiacum to fat-containing diets, which were then treated to induce rancidity, would enhance reproduction by delaying the development of rancidity. They found reproduction to be greater when the diets of rats were protected against development of rancidity by guaiacum.

IS GUAIAECUM NONTOXIC?

Taking advantage of this anti-oxidant action of guaiacum would mean its widespread use in foods. Before this can be done safely,

¹ Aided by a grant from Swift and Company.

however, it is necessary to establish beyond all reasonable doubt that the material is nontoxic. The extensive experiments reported here were directed toward answering this question: Is guaiacum nontoxic and therefore safe to use in food products, such as lard, which otherwise readily undergo spoilage from oxidative rancidity with consequent possible destruction of vitamin E? Of course, the extensive use of guaiacum in medicine in the past constitutes some evidence of nontoxicity, for great quantities of the material must have been ingested, apparently with impunity. However, more accurate and controlled data upon this subject was desirable.

The first possible point of action of guaiacum taken by mouth would be upon the gut itself—upon its digestive functions, its motility, its gross and histological appearance. Experiments aimed at discovering any such effects were carried out upon several species of mammals. Next we sought to find out the fate of guaiacum which is ingested. How long does it remain in the gut? Is it destroyed here? Does any of it get into the blood stream? These studies were largely chemical in nature and must be interpreted with caution, because the method used for quantitative detection of guaiacum was not entirely satisfactory. Furthermore, it is conceivable that though guaiacum itself may not be absorbed, some injurious component of it, which fails to give the characteristic chemical reaction for guaiacum itself, may be.

More emphasis was placed, therefore, upon the biological tests. Extensive experiments were conducted to test whether the animal organism as a whole is injured in any way by guaiacum ingestion whether it be from the guaiacum itself or from any conceivable fraction or disintegration product. The maintenance of weight of adults, the growth of young animals, the nutritive value of lard to which guaiacum has been added, the blood picture, reproductive virility, longevity, and kidney function were investigated. This was done chiefly upon rats, dogs, and cats. Finally, as many of these indices of well-being as possible and the influence of guaiacum ingestion upon them, were studied upon human subjects.

It should be emphasized that in all the work extremely large quantities of guaiacum were fed, as well as quantities which were adequate to prevent rancidity. In using these pharmacological doses it was hoped that any possible slight untoward effects might be brought to light.

ACTION OF GUAIAECUM IN THE GUT

Digestion and Utilization of Fats Containing Guaiacum: *In vitro* experiments were performed in which lard containing varying quantities of the anti-oxidant were digested with a bile salt-pancreatic

lipase mixture. Preliminary experiments by Davis (1936) led to the adoption of this procedure. To 30 grams of lard in a 125-c.c. Erlenmeyer flask 20 c.c. of .5 per cent bile salt solution was added. This was incubated at 38°C.(100.4°F.) for 20 minutes in an electric shaker. To the emulsion .5 gram of Wilson's lipase was added. This was rotated in the incubator at 38°C. for either 6 or 12 hours. In all experiments samples of lard containing varying quantities of guaiacum were run simultaneously with a control. After hydrolysis, the material was extracted with benzene and titrated for its fatty-acid content. From the results the percentage of fat digested could readily be computed. It should be noted that though the tests were carried out *in vitro* the attempt was made to simulate body condi-

TABLE 1

*In Vitro Digestion of Lard With Varying Quantities of Guaiacum Incubated With Bile Salts and Pancreatic Lipase for Periods of 6 and 12 Hours*¹

Incubation period	6 hours	12 hours
Guaiacum in lard tested	Digested	Digested
pct.	pct.	pct.
0.000	17.59 ± .47	22.46 ± .18
0.025	17.34 ± .46	22.36 ± .18
0.250	17.12 ± .51	22.31 ± .17
2.500	17.30 ± .49	22.44 ± .22

¹ Each figure is the average (with probable error) of 15 analyses.

tions as regards temperature, fat emulsification, and gut motility as well as reaction and enzyme action. The results (Table 1) analyzed statistically indicate that adding up to 50 times as much guaiacum as is necessary to prevent oxidative rancidity does not interfere with such *in vitro* hydrolysis. Even the controls do not show complete hydrolysis, of course, because of well-known reversibility of the reaction in which fats are hydrolyzed, according to Dietz (1907). An equilibrium is soon reached in which esterification proceeds as rapidly as hydrolysis.

Other experiments were aimed at getting indirect evidence of digestibility *in vivo*. Four groups of 10 rats each were fed identical diets. To the fat of the diet, varying quantities of guaiacum were added as follows: Diet 1, none; Diet 2, .05 gm.; Diet 3, .5 gm.; Diet 4, 5 gm. per 100 grams of lard, which constituted one-tenth of the diet by weight. Analyses by the method of Saxon (1914), slightly modified, were then made of the fat content of the feces as a measure of digestibility. The fat which passed into the feces

(unused fat) would presumably be that proportion which failed to be digested and absorbed.¹

Analyses showed that the following percentages of fat fed were not used and appeared in the feces:

- Diet 1—No guaiacum (controls)—.65 \pm .07%;
- Diet 2—.05 gm. guaiacum per 100 gm. lard—.67 \pm .06%;
- Diet 3—.5 gm. guaiacum per 100 gm. lard—.88 \pm .06%;
- Diet 4— 5 gm. guaiacum per 100 gm. lard—1.26 \pm .08%.

These figures are based upon eight duplicate analyses of three- or six-day collections of feces in each group. Only in the rats of Diet 4 is there a statistically significant increase in fat excretion. The lard in the diet of these rats, however, contained 100 times as much guaiacum as is necessary to prevent oxidative rancidity. The increase was probably of little or no physiological significance to the rats of this group, as indicated by observations on the growth of these animals, which will be discussed later.

Similar observations were made upon dogs. Four dogs were fed daily for 12 weeks on a diet containing 48 grams of fat. One week the dogs received no guaiacum and the next week they received one gram of guaiacum daily, mixed with and partially dissolved in the fat of the diet. During four days of each week the feces were collected and analyzed for fat. Since the quantity of fat ingested over this period was known, the quantity not utilized could be calculated. During control weeks (48 duplicate analyses) the dogs excreted 3.73 \pm .17 per cent of the fat they ate. During weeks of guaiacum feeding (36 duplicate analyses) the dogs excreted 4.92 \pm .17 per cent of the fat fed. These differences are only on the border line of statistical significance despite the fact that the animals were receiving 40 times as much guaiacum per 100 grams of fat as is necessary to prevent oxidative rancidity of lard.

Water Content of Feces: The 40 rats, whose fecal excretion was described above, were studied for a possible cathartic action of guaiacum. This inert material might stimulate gastro-intestinal motility or might mechanically or chemically irritate the mucosa. In either case, one should expect an effect upon the consistency of the stools. These were therefore analyzed for water content. The result showed the following percentages of moisture in the stools, based upon eight

¹ The significance of these experiments is not affected by the recent work of Krakower (1934) which indicates that a large percentage of the fecal fat in human beings represents not unabsorbed fat but fat excreted into the intestine. Krakower's work seems to show that a relatively constant amount of fat in any individual is excreted daily. Granting this, any significant change in the digestion and absorption of fat from guaiacum ingestion should still change the total quantity appearing in the feces.

duplicate analyses of three- or six-day collections of feces in each group:

- Diet 1—No guaiacum (controls)— $24.4 \pm 1.3\%$;
- Diet 2—.05 gm. guaiacum per 100 gm. lard— $22.3 \pm .68\%$;
- Diet 3—.5 gm. guaiacum per 100 gm. lard— $27.2 \pm .66\%$;
- Diet 4— 5 gm. guaiacum per 100 gm. lard— $34.4 \pm .89\%$.

The figure for the rats of Diet 4 is significantly greater than that for the controls, indicating that in these high concentrations (100 times that necessary to prevent rancidity) some cathartic action is produced on rats. No significant effect in this direction was observed in the rats on Diet 3, however, even though the lard in the diet of these animals contained ten times as much guaiacum as is necessary to prevent rancidity.

Fecal water content was also studied on seven dogs. Two controls received no guaiacum, three received .5 gram daily, and two received 1 gram daily. Otherwise, the diets were the same. In each group, more than 20 duplicate analyses of 24- or 48-hour fecal samples were made. The results, expressed in percentage of dry matter in the feces, are as follows:

- Dogs fed no guaiacum— $38.2 \pm .61\%$;
- Dogs fed .5 gm. guaiacum per day— $41.5 \pm .92\%$;
- Dogs fed 1 gm. guaiacum per day— $41.0 \pm .80\%$.

The figures, statistically not significant, show that the ingestion of as much as a gram of guaiacum per day had no influence upon the water content of the stools of dogs.

Gross and Microscopic Appearance of Gastro-Intestinal Mucosa: The gastric and intestinal mucosa of all rats, cats, and dogs used in these experiments were examined at autopsy, both grossly and in some cases histologically, and in no instance was there any hyperemia, ulceration, or any other evidence of chronic irritation.

FATE OF GUAIAECUM IN THE ORGANISM

Recovery of Guaiacum Fed: The work on this subject is based upon a quantitative test for guaiacum that is not so accurate as one would desire. The test depends upon the development of a blue coloration when guaiacum plus blood plus H_2O_2 are brought together. The dilution at which the color persists for at least one minute is used in the quantitative estimation and is only accurate within about 50 per cent.

In a group of six dogs varying quantities of solid guaiacum were fed (without food), and after periods of from 10 to 24 hours the animals were killed; the feces and the contents of the stomach, duo-

denum, small intestine, and large intestine were analyzed for guaiacum. In this short series 83 per cent of the material fed was recovered, distributed as follows: from the stomach, 58.5 per cent of the amount fed; from the duodenum, .3 per cent; from the small intestine, 6 per cent; from the large intestine, 12.5 per cent; from the feces passed, 6 per cent. This tends to show that the material may remain in the stomach for a long time, and that probably little if any is absorbed.

Experiments more physiological in nature were done in which single large doses of guaiacum were mixed with food and fed to dogs. On successive days thereafter feces were collected and analyzed in an attempt to determine how much of the guaiacum passed through the gastro-intestinal tract and how soon it did so. In four dogs fed 20 or 40 grams and one fed two grams it took from two to four days for the feces to become guaiacum-free. Here again we encounter the slow passage of the material through the alimentary canal. The amounts recovered in the feces ranged from 67 to 99 per cent of that which was fed.

Also, feces were collected on four dogs fed .5 gram or 1 gram of guaiacum daily for periods varying from nine to 18 days. It was found here that the feces were guaiacum-free on many days, sometimes for two or three successive days, and that relatively large amounts would be excreted on some days. Quantitative estimates of the total amount of guaiacum recovered in all these experiments were not very consistent, but the results indicated that on the average approximately one-half of the amount ingested does not appear in the feces.

Fate of Guaiacum Not Recovered: What happens to that guaiacum which fails to appear in the feces? Is it destroyed by the digestive juices? Does it disintegrate in the colon, mixed with feces? To test the former possibility guaiacum has been subjected to the action of the digestive juices and of feces *in vitro*. In 22 experiments one-half gram, one gram, or two grams of guaiacum were exposed to the action of dog gastric juice for four, five, or eight hours in a shaker kept at 38°C. (100.4°F.). In every experiment all the guaiacum, within the limits of accuracy of the test, was recovered in the residue. None of it went into solution, and none of it was destroyed. One would expect this result in view of the fact that guaiacum is not soluble in acid solutions.

Upon exposure to artificial pancreatic juice in the same manner (14 experiments) little if any of the material went into solution or was destroyed by the juice. Upon including bile in the mixture

approximately a tenth of the guaiacum went into solution, but there was no definite indication of destruction of the material.

On the other hand, appreciable quantities of guaiacum are apparently destroyed in the colon. At any rate, after adding known quantities of guaiacum to feces *in vitro*, it was possible to recover only a small fraction of the guaiacum after incubation at body temperature for 24 hours, in some instances.

The possibility remains, nevertheless, that some of the material is absorbed into the blood stream. We have found that normal blood and urine give negative tests for guaiacum by our method and that after addition of guaiacum *in vitro* these fluids give positive tests. Twelve dogs were fed two to four grams of guaiacum at one time and were killed at intervals of from four to 17 hours. The whole blood was examined for guaiacum. In 11 instances none was found by our tests; in one instance 7.5 mgm. were found in 500 c.c. of blood. When guaiacum is injected directly into the blood, it rapidly disappears; in seven dogs 9 mgm. was the most guaiacum found five hours after intravenous injection of 300 to 400 mgm.

Body fat taken from subcutaneous and mesenteric deposits of 11 dogs and eight cats fed guaiacum many months gave negative tests for guaiacum in every instance.

In no instance (12 tests) did the feeding of guaiacum (two to four grams) to dogs lead to a positive test for the material in the urine. When the substance is directly injected into the blood stream, however, the urine becomes positive for guaiacum in some instances. In 17 of a total of 19 intravenous injections (of 300 to 600 mgm.), from 0 to 10 per cent of the quantity injected was recovered from the urine. In two instances 30 and 100 per cent were recovered.

The obvious objection to these blood studies is that inasmuch as guaiacum is a mixture and our test may indicate the presence only of one component, we have no evidence about the absorption of other components, which may be toxic. Therefore, tests were made of the effects of intravenous injections in unanaesthetized dogs. A total of 31 injections of .2 to .8 gram each were made upon six dogs. No acute or delayed untoward effects were obtained, except for alcoholic intoxication owing to the alcohol used as a solvent for guaiacum. Control animals receiving alcohol alone, intravenously of course, displayed the same symptoms.

We see from this series of experiments that much of the guaiacum fed passes out in the feces, that an appreciable quantity may be destroyed in the colon, that little or none of it is absorbed, and that even if it should be absorbed it apparently is nontoxic. Attention is again called to the very large doses employed in these experiments.

It was felt of course that these acute experiments were not adequate in themselves. Prolonged experiments were necessary in which as many criteria of general physiological well-being as possible should be used without relying solely upon actual chemical tests. These experiments are described below.

EFFECT OF GUAIAECUM UPON BODY WEIGHT, BLOOD PICTURE, AND GROWTH

Weight Maintenance of Dogs and Cats, and Blood Counts Upon Dogs: The effect of guaiacum ingestion upon body-weight maintenance was studied in 11 full-grown dogs fed a standard diet consisting of a mixture of beef lung, 300 grams; white bread, 300 grams; yeast, 8 grams; bone meal, 45 grams; and cod liver oil, 1 teaspoonful daily, for 62 to 103 weeks. Three control animals received no guaiacum, five dogs got from .5 to 1 gram daily, and three got 1 gram daily throughout. Every dog except one not only maintained his weight but actually gained. The one exception was a dog weighing 19 kilograms which was fed one gram of guaiacum daily. This dog lost two kilograms in 75 weeks, which seems insignificant. The general behavior and appearance of all dogs were quite normal in all respects. Red-cell counts, white-cell counts, and hemoglobin determinations made three times upon each of the 11 dogs at intervals during the experiment failed to reveal any deviations from normal in any case.

A similar experiment was conducted upon eight full-grown cats for 34 to 117 weeks. The cats received the same diet as the above-mentioned dogs. Three received no guaiacum and five were fed .5 to 1 gram of guaiacum daily. Only one cat in the group receiving 1 gram of guaiacum daily failed to gain weight. None was apparently adversely affected by the large quantities of guaiacum ingested.

The dogs received daily amounts of guaiacum up to .1 gram per kilogram of weight, to receive which a man weighing 60 kilograms would have to eat 12,000 grams of lard (containing .05 per cent guaiacum) daily. The dosage fed the cats was even higher, approximating .66 gram per kilogram.

Nutritive Value of Lard Containing Guaiacum: In the above-described experiments on body weight of cats and dogs, as well as in the growth experiments on rats to be described in the next section, the objection might be raised that the different animals, fed *ad libitum*, may have eaten different quantities of food and that, therefore, any deficiency in the nutritive value of lard containing guaiacum might be masked by the guaiacum-fed animals simply eating more food. The following experiment was devised to test more rigorously the

relative nutritive value of lard containing guaiacum as compared with lard free from guaiacum.

Forty rats were divided into four groups and fed the following basal diet: casein, 18 per cent; starch, 48 per cent; yeast, 5 per cent; wheat germ, 3 per cent; inorganic salts, 4 per cent; agar, 2 per cent; lard, 10 per cent; butter, 10 per cent. Group 1 received

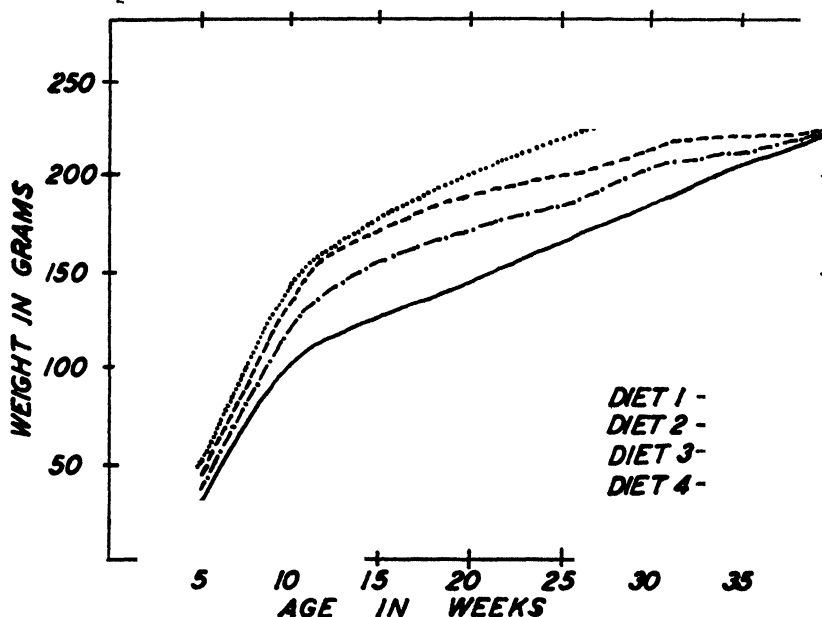


FIG. 1. Growth curves of four groups of ten rats each. The rats in each group were fed the same number of grams of the same basal diet per kilogram of rat per day. The nutritive value of the basal diet plus guaiacum was thus compared with that of the basal diet alone.

nothing else; Group 2 received .05 gram of guaiacum per 100 grams of lard; Group 3, .5 gram of guaiacum per 100 grams of lard; and Group 4, 5 grams per 100 grams of lard. Diet 4 thus contained 100 times as much guaiacum per 100 grams of lard as is needed to prevent rancidity. The rats were fed this for 41 weeks. The feeding was done by the method of Still and Koch (1928), which insures a minimum of scattering of food as well as the ingestion by each group of rats of the same amount of food per gram of body weight per day. Thus no variation in rate of growth can be attributed to one group eating more food than another. This method also provides for the rats getting a little less food than the optimum for a maximum rate of growth. Any deficiency in any diet would thus show up. The growth curves are plotted (Fig. 1); if there is any significant difference here, it is in favor of the groups getting guaiacum. At

least the indication is that lard containing guaiacum is quite as adequate in contributing to rat growth as is lard without guaiacum. In this experiment, of course, the results indicate that the digestion, absorption, and utilization of proteins and carbohydrates as well as fats, is not interfered with.

Growth in Three Generations of Rats: In this experiment rats were submitted to an extremely rigid test of possible toxicity of guaiacum by addition of large quantities of the gum to an otherwise adequate diet and extending the observations over three generations. Forty rats were divided into four groups and fed the same basal diet as that of the rats in the preceding experiment. Then, in addition, the following quantities of guaiacum were fed, mixed with and dissolved in the lard of the basal diet:

- Diet 1—None (control group);
- Diet 2—.05 gm. per 100 gm. of lard;
- Diet 3— .5 gm. per 100 gm. of lard;
- Diet 4— 5 gm. per 100 gm. of lard.

Diet 2 contained just the quantity of guaiacum necessary to prevent oxidative rancidity. Diet 4 contained 100 times as much guaiacum as this. Throughout the lifetime of these rats and for three successive generations they ate approximately .2 gram of guaiacum daily per kilogram of body weight. A 60-kilogram man would have to eat 24 kilograms of guaiacum-treated lard per day to ingest this much guaiacum. This is over 400 times the per capita consumption of lard, shortening, and margarine in the United States.¹ The feeding of these tremendous quantities of guaiacum should reveal the presence of even mild toxicological effects.

The second and third generation descendants (80 in number) of the original rats were maintained throughout their lifetime on the same diet as their parents. The usual care was taken with regard to cleanliness of rats, cages, and bedding. The growth curves of the rats of all three generations are shown (Fig. 2) over the normal growth period of 40 weeks. The curves for rats on all four diets lie remarkably close together. At no time does any one curve differ from any other by more than 25 grams.

For comparison, Fig. 2 includes the growth curves of normal rats, as given by Donaldson (1924). This curve lies below all the curves for our rats, indicating clearly that normal growth occurred in the rats on all of our diets.

¹ According to Swift and Company's data the average per capita consumption of these fats is 55 grams per day.

REPRODUCTION IN THREE GENERATIONS OF RATS

Reproductive virility was determined for the rats used in the preceding experiment by observations upon the number of pregnancies, number of young born, and number of young weaned (Table 2). The time unit in terms of which pregnancies, etc., are expressed is called the "adult female rat weeks." The number of adult females observed multiplied by the number of weeks they were kept with adult males on the same diet equals the number of such rat weeks; for example, 100 rat weeks would mean 10 female rats

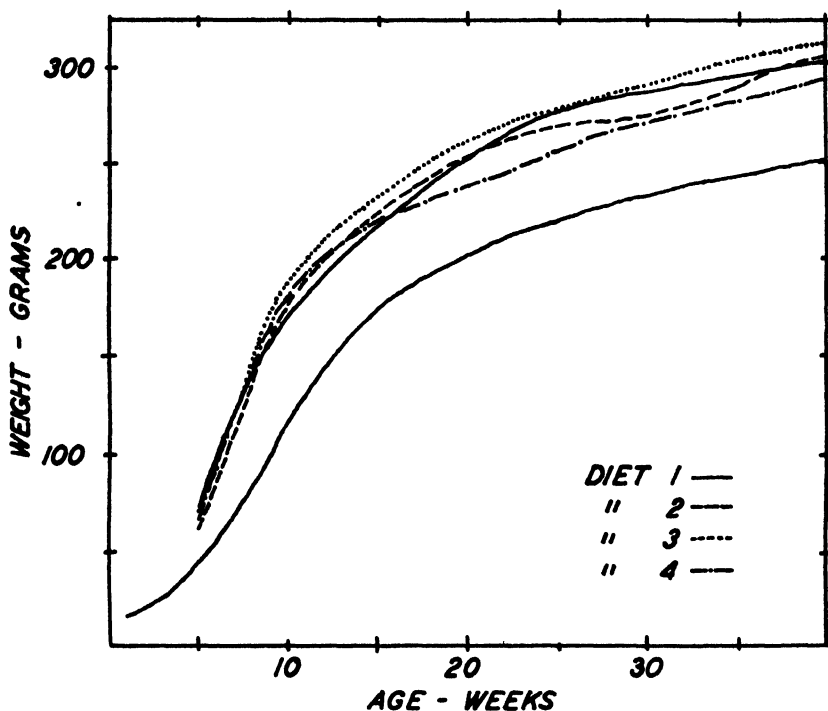


FIG. 2. Growth curves of 120 rats of all three generations. The lower curve (solid line) is based upon data given by Donaldson (1924).

observed for 10 weeks, or 12 female rats observed for 8 plus weeks. Special attention should be paid the third, fifth, and seventh columns (Table 2). Column 3 is a comparison of the average interval elapsing between successive pregnancies. It is an expression of the rate at which pregnancies occurred on the various diets and in successive generations. In this regard, the performance of rats on all three guaiacum-containing diets was about equal to, or better than, that of rats on the control diet, even if the results on the third generation of the control diet, which were poor, are disregarded.

The average number of young born per pregnancy (Column 5) is slightly less in the Diet-4 rats (most guaiacum) than in the control group. The performance of rats on Diet 3, however, equaled that of the control group, though Diet 3 contained 10 times as much guaiacum per 100 grams of lard as is necessary to prevent oxidative rancidity.

TABLE 2
Fecundity of Three Generations of Rats on Diets Containing Varying Quantities of Guaiacum

Column		1	2	3	4	5	6	7
Diet	Generation	Total adult female rat weeks	Total number pregnancies	Average interval between pregnancies (weeks)	Total young born	Young per pregnancy	Total young weaned (21 days)	Per cent weaned
1	1	226	29	7.8	192	6.6	157	82
	2	211	18	11.7	146	8.1	139	95
	3	103	6	17.2	52	8.7	31	60
	1-3	540	53	10.2	390	7.4	327	84
2	1	218	25	8.7	165	6.6	144	87
	2	219	24	9.1	168	7.0	145	86
	3	218	28	7.8	172	6.1	120	70
	1-3	655	77	8.5	505	6.6	409	81
3	1	223	27	8.4	202	7.5	179	89
	2	215	31	6.9	233	7.5	207	89
	3	212	18	11.8	137	7.6	112	82
	1-3	653	76	8.6	572	7.5	498	87
4	1	224	23	9.7	155	6.7	126	81
	2	216	21	10.3	135	6.4	115	85
	3	196	27	7.3	158	5.9	123	78
	1-3	636	71	9.0	448	6.3	364	81
Grand total.....		2,484	277	9.0	1,915	6.9	1,598	83
Published norms.....		11.8 ¹	7.0 ¹	86 ¹
								78 ²

¹ Donaldson (1924). ² Evans and Bishop (1923).

Column 7 of the table shows only very slight, insignificant differences in the percentage of new-born rats which survived the period of weaning.

The last line of the table is given to show that the fecundity of all the rats used in the experiment compares favorably with published data on large series of normal rats as regards frequency of pregnancies,¹ number of young per litter, and proportion of young

¹ The figure, 11.8, for the average interval between pregnancies, is not strictly comparable to ours, of 9 weeks. It is computed from the following data from Donaldson: "Reproductive period in the rat equals 65 weeks; average number of litters during this period, per rat, equals 5.5."

born which were successfully weaned. Also, the average number of pregnancies per rat was identical, at 5.5, in our series and as given by Donaldson (1924).

By these tests of reproductive activity, Diet-4 rats (most guaiacum) compare quite favorably with the controls. The rats on Diet 3 are, if anything, slightly superior to the control rats. The litters of this group were dropped more frequently, and a slightly larger percentage of the young were weaned.

The data indicate that guaiacum produces no deleterious effects upon reproduction when fed in large quantities over a period of three generations of rats.

LONGEVITY

Including all animals the average life spans for rats of all three generations were as follows: Diet 1 (controls), 62.4 ± 3.9 weeks; Diet 2, 79 ± 4.6 weeks; Diet 3, 77.5 ± 5 weeks; Diet 4 (most guaiacum), 72.8 ± 4.5 weeks. The differences in these figures are not statistically significant. When those rats which died within the first year are excluded, average longevity figures are as follows: Diet 1 (controls), 89 weeks; Diet 2, 100 weeks; Diet 3, 100 weeks; Diet 4 (most guaiacum), 93 weeks. According to Donaldson, these rat ages would correspond to ages in man of approximately 53, 60, 60, and 56 years, respectively.

The four individual rats in the entire series which lived longest included one representative from each diet as follows: Diet 1 (control), 147 weeks (equals 88 years in man); Diet 2, 145 weeks (87 years); Diet 3, 141 weeks (85 years); Diet 4 (most guaiacum), 143 weeks (86 years).

These data may be compared favorably with figures given by Donaldson, who states that rats rarely live 170 weeks and that they tend to become weak and decrepit at 100 weeks.

Again, there is no apparent effect upon the life span from feeding large quantities of guaiacum.

MICROSCOPIC FINDINGS

Microscopic sections were made of the kidney, liver, spleen, and lungs of six control rats and 17 rats on the guaiacum diets. In no tissue was there evidence of chronic damage. Particular attention was paid to the liver and kidney, where chronic intoxication might be expected to leave histological evidence. None was found. Two of the guaiacum-fed rats showed slight glomerular damage to a degree judged insufficient to produce impaired function. In any case, the damage could not be ascribed to guaiacum, because most of the

rats fed guaiacum were free from such change, and also, the same mild lesion was observed in one of the controls.

A number of the rats showed small areas of focal necrosis of cells in the liver. This condition, of unknown etiology, was present in the livers of all the controls examined, so that it cannot be ascribed to guaiacum ingestion.

Most of the animals showed changes in the lungs, indicating that pulmonary infection was the cause of death; edema, hemorrhage, bronchitis, broncho-pneumonia, and even gangrene were common. The second most common immediate cause of death was an ailment of undetermined etiology associated with a terminal diarrhea, affecting control and guaiacum rats alike. There were no consistent histological changes in these animals.

Careful examination of the intestine for evidences of irritation of the mucosa by guaiacum was considered to be important. The gastro-intestinal mucosa is the only tissue with which we can be certain ingested guaiacum comes into contact. The histological preparations of rat intestine were unsatisfactory for this study because most of the rats, allowed to die eventually from "natural" causes, could not be autopsied immediately after death. Consequently, post-mortem autolysis of the intestinal mucosa was extensive. Therefore, histological findings obtained on dogs and cats were considered especially significant. Histological sections from three dogs (fed 1 gram of guaiacum daily for 75 weeks) and from two cats (fed .5 to 1 gram of guaiacum daily for 74 weeks), which were killed and autopsied at once, showed a perfectly normal intestinal mucosa in each case, with no suggestion of irritation or injury.

Gross and histological examination of the lungs, kidneys, livers, and spleens from these dogs and cats also revealed normal organs.

EXPERIMENTS ON HUMAN SUBJECTS

Effect of Large Single Doses of Guaiacum: It was considered desirable to extend as many of these observations as possible to human subjects. In all, six human subjects took a total of ten doses of two or three grams of guaiacum at one time. Three grams of guaiacum are equivalent to the amount found in 6,000 grams of guaiacum-lard. On the basis of the per capita daily lard consumption of about 30 grams, this is a six-months' supply. The only untoward action from these large quantities was that one or two loose stools were passed after the ingestion in some instances.

Effect of Daily Ingestion for Two Years: Eleven graduate students and staff members (four women and seven men) ingested .05 or .10 gram of guaiacum daily for periods of 18 to 104 weeks. Five

subjects continued the experiment over 90 weeks. The subjects were on a normal, adequate diet for the whole period. The guaiacum ingested was mixed into little pellets of chocolate. The amounts taken daily are equivalent to that which would be ingested in 100 or 200 grams of lard containing guaiacum in a concentration of .05 gram per 100 grams of lard. These amounts were chosen so as to be well in excess of the per capita daily consumption of lard. Lusk (1928) quotes data of Voit and Rubner on the total fat content of normal dietaries. These figures vary from 46 grams per day for a man weighing 70 kilograms doing light work to 100 grams for a man doing heavy work. Lusk also cites data from Rubner, giving 31 to 65 grams as the per capita daily total fat consumption in four European cities, based on municipal statistics. Inasmuch as these figures are for total fat ingested, we may be sure that the consumption of lard and all lard products would be well under this figure.

Red and white blood-cell counts and blood-hemoglobin determinations were made monthly. Also, each month Fishberg's (1930) modification of Volhard's urine-concentration test for kidney function was performed on each subject. Observations were also made of body weight, number and consistency of stools, general physical condition, and subjective effects.

The red blood-cell counts are plotted, giving the highest and lowest count observed each month in any subject as well as the average for the group of 11 subjects (Fig. 3). The hemoglobin determinations all fell within the normal range of 80 to 100 per cent except for two observations, one of which was 70 per cent, another 75 per cent.

The white blood-cell counts show the range within which all counts fell as well as the average for the group (Fig. 3). It is clear that both the red and white counts fall within the normal range throughout the experiment and that no significant trend in either direction occurred.

None of the subjects either lost or gained significant amounts during the experiment.

Results of studies on the effect of guaiacum ingestion upon number of bowel movements give the average number of stools per week for each month of the experiment, including the range as well as the average for the group (Fig. 4). The curves show no significant trend throughout the period of observation.

That the figures throughout are within the normal limits is indicated by comparison with control data derived from a dietary experiment performed in our laboratory several years ago. At that time it was found that for a ten-week period, 14 subjects passed

an average of 10.5 stools per week, as compared with an average of 9.5 stools in the 11 subjects of this experiment. Two of our subjects took part in both experiments. M. J. had an average of 7 stools a

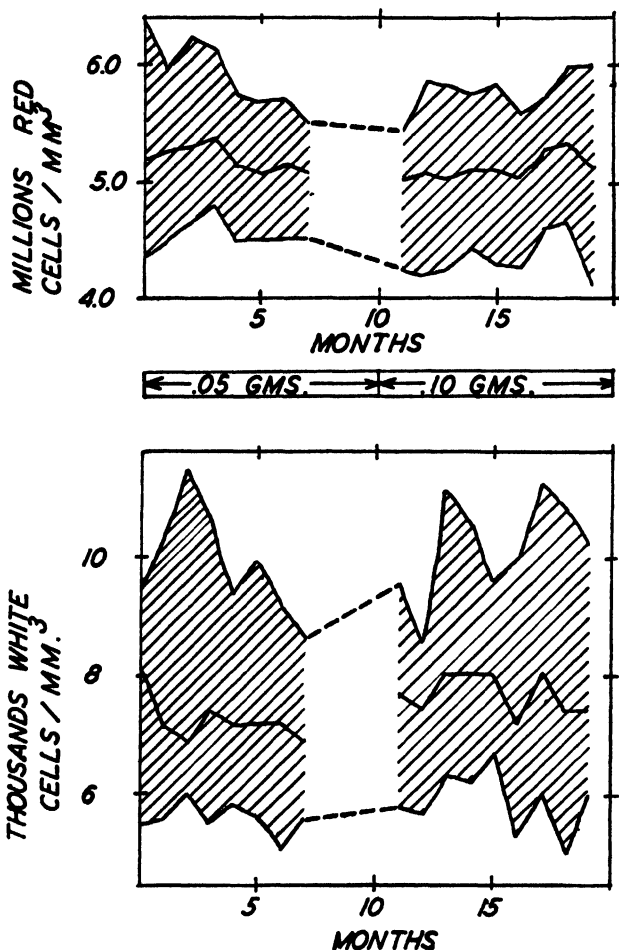


FIG. 3. Red (above) and white (below) blood-cell counts of 11 human subjects ingesting .05 or .10 gram of guaiacum per day.

Upper line: Highest figure observed in any subject each month.

Lower line: Lowest figure observed in any subject each month.

Middle line: Average for the entire group of 11 subjects.

Shaded area: Range within which all observations fell.

Dotted line: No observations made during this period.

week in the former (control) experiment and 7.3 during guaiacum ingestion. V. J. had 8.1 stools in the control period and 7.2 during guaiacum ingestion. As it was also observed in the present experiment, that no regular change occurred in the consistency of the

stools, it seems clear that the relatively large daily doses of guaiacum had no ill effect upon colon motility.

The monthly urine-concentration tests were all negative, none of the observations being outside the range for normal kidney function.

No subjective effects, adverse or otherwise, which might be attributed to the guaiacum were noted. The general well-being of all subjects has been unimpaired, although some individuals had the occasional colds to which all are subject.

Fat Utilization and Excretion: An experiment similar to that done upon dogs was carried out on four adult human subjects (three males and one female) on a carefully controlled diet. These indi-

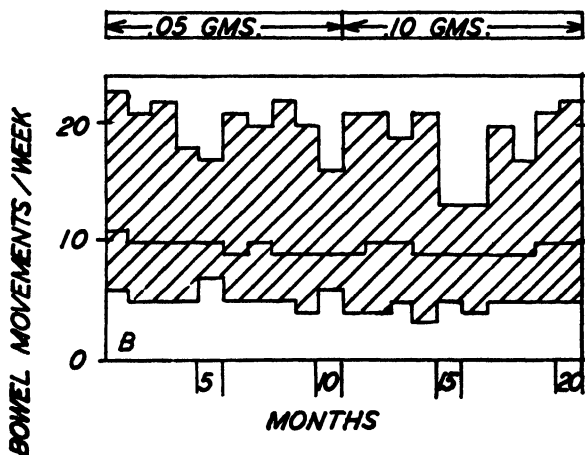


FIG. 4. Number of bowel movements per week for 11 human subjects ingesting .05 or .10 gram of guaiacum per day.

Upper line: Highest figure observed in any subject.

Lower line: Lowest figure observed in any subject.

Middle line: Average for entire group of 11 subjects.

Shaded area: Range within which all observations fell.

viduals ate weighed quantities of food of known composition over a period of eight to 12 weeks. The diet was balanced in all respects and was planned to have a rather high fat content. The daily fat intake varied from 300 grams in some individuals to 600 grams in others. To rule out errors owing to possible inexact knowledge of the fat content of the foods eaten, the following dietary régime was adopted. During the first (control) week an accurate record of the quantity of all materials ingested was kept. During the following week each meal was an exact duplicate, qualitatively and quantitatively, of the corresponding meal of the previous control week. Also, during the second week 100 mgm. of guaiacum per day were taken, dissolved in the daily milk-cream mixture which constituted

the largest single source of fat in the diet. If the diet was changed slightly the third (control) week, the same change was made during the fourth (guaiacum-ingestion) week, etc.

Before the first meal and after the last meal of each four-day period, powdered charcoal was eaten to mark off the feces of the experimental period. The feces were collected for the whole four-day period and analyzed for fat.

The data show that during the control weeks (21 duplicate determinations) the subjects excreted $2.55 \pm .07$ per cent of the fat fed. During the guaiacum-ingested weeks (20 duplicate determinations) $2.76 \pm .10$ per cent of the fat fed was not used. The difference is statistically insignificant. These figures correspond very well with the results of Smith, Miller, and Hawk (1915) who determined that in normal humans, 96 to 100 per cent of fed lard was digested and absorbed.

Our observations on human beings in the chronic experiment indicate that no tendency toward diarrhea is induced by guaiacum ingestion. A further check on this was made in the carefully controlled human dietary experiment reported here by determining the water content of the feces as well as the fat content. During guaiacum-ingestion periods the feces contained $80.7 \pm .8$ per cent moisture as compared with $79.7 \pm .8$ per cent during control periods.

SUMMARY AND CONCLUSIONS

The above extensive work, carried out upon rats, dogs, cats, and human beings over a period of four years, has demonstrated the following with regard to the ingestion of guaiacum in the quantities indicated in the test.

1. In the alimentary canal guaiacum exerts no irritating action, and only in far greater quantities than would ever be ingested is there slight cathartic effect. These results were obtained by analyses of the water content of the feces in experiments on seven dogs, 40 rats, and four human beings and by records of number of weekly bowel movements in 14 human beings during a two-year ingestion experiment as well as by anatomical (gross and microscopic) observations on rats, cats, and dogs.

2. *In vitro* studies testing the digestibility of lard by pancreatic lipase plus bile (161 tests) indicated that addition of guaiacum to the lard had no effect upon the hydrolysis.

3. *In vivo* studies upon the content of unused fat excreted in the feces of 40 rats, seven dogs, and four human beings confirmed the *in vitro* studies indicating the lack of any impairment of fat digestibility or absorption as a result of the presence of guaiacum in lard.

4. Numerous acute and chronic experiments on rats, dogs, and man indicated that ingested guaiacum may remain in the alimentary canal several days and that most of it appears in the feces. *In vitro* studies demonstrated that the guaiacum which failed to be excreted was probably destroyed or changed in the colon. Gastric and pancreatic digestion did not destroy guaiacum. Very little if any of the material is absorbed into the blood. Acute experiments (31) on intravenous ingestion in dogs indicate that the presence of guaiacum in the blood is not injurious.

5. Experiments on 11 dogs, eight cats, and 14 human beings ingesting guaiacum for one to two years showed no effect upon body weight.

6. The blood picture (red and white cell numbers and hemoglobin concentration) of seven dogs and 14 human beings was unaffected by eating guaiacum over long periods of time.

7. The kidney function of 14 human subjects was unchanged during one to two years of daily guaiacum ingestion.

8. Experiments on 40 growing rats over a period of 40 weeks showed that gram for gram lard containing more than enough guaiacum to prevent rancidity is of as great nutritive value as lard free from guaiacum.

9. Growth, reproduction, and longevity of three generations of rats (120 in all) were unaffected by ingesting 100 times as much guaiacum as was necessary to prevent rancidity of the lard fed.

10. Studies upon the microscopic appearance of the intestines, liver, spleen, and kidney of 23 rats, five cats, and seven dogs, and upon the gross appearance of the organs of many other rats revealed that no pathology was caused by prolonged ingestion of large quantities of guaiacum.

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UTILIZATION OF CALCIUM OF SPINACH AND KALE

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In previous studies on rats by Fincke and Sherman (1935) it was found that the calcium of kale was practically as well utilized by the animal body as that of milk, while the calcium of spinach was utilized poorly if at all. This could not have been due to the presence of fiber, as the same results were obtained when the fiber contents of all three diets were equalized. However, it did appear to be due, in part at least, to the presence of oxalate in the spinach.

Several other investigations have been conducted on utilization of the calcium of vegetables. McClugage and Mendel (1918) reported that the calcium of spinach and carrots was poorly utilized by dogs. Rose (1920) studied the calcium retentions of young women on diets in which 55 per cent of the calcium was provided by carrots, the total calcium intake being close to the estimated minimum requirements for maintenance. Positive calcium balances were obtained in all cases but one, and in that case the losses were small and were traceable to a digestive upset.

Sherman and Hawley (1922), in comparing the storage of calcium by children receiving different amounts of milk, found that larger retentions always occurred with a quart of milk than with a pint. However, when a pint of milk was fed together with sufficient carrots, spinach, and celery to provide additional calcium equivalent to that present in another pint of milk, the storage was not as great as with the quart of milk. Rose and MacLeod (1923) showed that when 73 per cent of the calcium of the diet came from almonds, it was well utilized by adult women, but that when the almonds supplied 85 per cent of the calcium, they were not so efficient a source of calcium.

McLaughlin (1927) concluded that the calcium of spinach was utilized by adult women. The total output of calcium on the diet in which spinach furnished 70 per cent of the calcium, however, was about one-sixth greater than on the diet containing milk as chief source of calcium, and consequently the retentions of calcium on the spinach diet were much smaller than on the milk diet.

Edelstein (1931), Edelstein, Langer, and Langstein (1932), and also Schlutz, Morse, and Oldham (1933) found that spinach lowered the retentions of calcium in babies. Mallon, Johnson, and Darby

(1933) reported good availability of the calcium of leaf lettuce for adults, and Adolph and Chen (1932), that of soy beans.

The present investigation was undertaken to extend the work previously done with rats in comparing the availability for humans of the calcium of two typical green leafy vegetables—spinach and kale. Spinach has been found by many investigators to contain relatively large amounts of oxalic acid; kale contains amounts too small to be measured by the ordinary methods. Kohman and Sanborn (1935) reported that while some of the calcium of calcium oxalate could be used for bone formation, it was of a low order of availability. Horwitt, Cowgill, and Mendel (1936) have recently reported that only 30 per cent of the calcium of spinach is rendered soluble by *in vitro* digestion. The studies here described consisted of calcium balance experiments on two healthy women on diets in which most of the calcium was supplied by either kale or spinach.

EXPERIMENTAL PROCEDURE

Two healthy women weighing respectively 62.7 and 58.2 kilograms, served as subjects in this investigation. Diets were planned in which kale or spinach was the chief source of calcium. With each diet nine-day experimental periods were used, in each case consisting of a three-day preliminary period to allow the body to become accustomed to the diet, followed by two three-day experimental periods during which feces and urine were collected. All food and excreta were analyzed for calcium, and retentions of calcium were determined.

The kale was the so-called "field kale," grown on one of the Oregon State College farms. It is of the same family as the ordinary curly or Scotch kale, but the leaves are smooth and somewhat coarse in texture but palatable and pleasing in flavor. The kale was cut fresh every two days and prepared by stripping the leaves from the tough midrib and petiole. It was then washed, rinsed in distilled water, and dried in the air until free of visible moisture but not wilted. This usually took one and a half to two hours.

The spinach for the first series was grown in California during the winter and was purchased in the local market. The spinach for the second spinach series was locally grown in the spring and likewise purchased in the market. The leaves were used without the stems and were washed, rinsed with distilled water, and air-dried. The individual portions of kale or spinach were boiled in a small amount of water until tender and then drained. The cooking liquid, together with water used to rinse the pans in which the vegetable and the meat were cooked, was drunk separately as a bouillon, so that none of the calcium was lost by dissolution or precipitation on the walls of the cooking vessel.

When the kale and spinach were to be kept overnight they were wrapped in paper and kept in the mechanical refrigerator at a temperature of 3.3°C. (38°F.). The diets as given are shown (Table 1). Distilled water was used exclusively for drinking, for the tea infusion, and for cooking. Whenever possible, a sufficient amount of one food was purchased at the beginning of the experimental period and used throughout, samples being taken for analysis. Ground lean round of beef was frozen in the freezing trays of a mechanical refrigerator.

As Diet 1 was probably low in vitamin B, a solution of crystalline vitamin B hydrochloride sufficient to provide about 300 Sherman

TABLE 1
Diets Containing Kale or Spinach as Chief Source of Calcium

Ingredients	Diet 1		Diet 2		Diet 3	
	Weight of food	Calcium	Weight of food	Calcium	Weight of food	Calcium
	gm.	gm.	gm.	gm.	gm.	gm.
Crackers, soda.....	158	.030	158	.030	158	.030
Butter.....	66	.007	66	.007	66	.007
Sugar.....	23	23	23
Beef, lean, ground.....	150	.010	150	.010	150	.015
Apple, cored and pared.....	150	.008	150	.008
Bacon.....	22	.001	22	.001	22	.001
Tomato juice.....	114	.005	114	.005	114	.005
NaCl, C.P.	3	3	3
Kale.....	134	.339
Spinach.....	540	.243	490	.387
Weak tea infusion.....	<i>ad lib.</i>	<i>ad lib.</i>	<i>ad lib.</i>
Total.....400304445
Total calories (approximate).....	1,755	1,823	1,717

units per day was taken. Diets 2 and 3, containing larger amounts of green vegetables, contained larger amounts of vitamin B.

Calcium analyses were made by a modification of McCrudden's method (1935). In Diet 1, 84.7 per cent of the calcium was provided by the kale; in Diet 2, 79.9 per cent by the spinach; while in Diet 3, 86.9 per cent came from the spinach. Both spinach and kale varied greatly in calcium content. Several preliminary lots of both kale and spinach were analyzed for calcium, and amounts were estimated from these determinations to provide a total intake of .45 gm. of calcium per day. This was the average daily minimum requirement which Sherman (1920) determined from a study of many balance experiments. When the kale and spinach which were actually eaten in

Diets 1 and 2 were analyzed, however, they were found to be unusually low in calcium, so that the average daily intakes of calcium were .400 gm. for the kale series and .338 gm. for the first spinach series. For this reason, another nine-day balance experiment was later conducted with spinach. In this case (Diet 3), the analyses of spinach were made beforehand, and sufficient spinach eaten to provide .387 gm. of calcium, so that the total daily ingestion of calcium was .445 gm.

The apple, which was included in Diets 1 and 2, was omitted from Diet 3 in order to avoid any possible deleterious effects of increased fiber on calcium retention. The fiber contents of Diets 1 and 3 were therefore approximately the same.

After the three-day preliminary period with each diet, feces and urine were collected for each three-day experimental period, the feces being marked off with carmine in the usual manner. The feces were dried on a steam bath and then in an electric oven at 100°C. (212°F.), weighed, and ground in a mortar. Samples were analyzed for calcium. The urine was measured in a graduated cylinder, thoroughly mixed, and 100-c.c. aliquots for analysis were drawn off by pipette. Toluene was used as a preservative for the urine.

DISCUSSION OF RESULTS

The results show that Subject A, on a diet containing 1.200 gm. of calcium during the three-day period, principally from kale, excreted 1.374 and 1.178 gm. in the two periods (Table 2). The calcium balances for these two periods were therefore $-.174$ and $+.022$ gm. respectively, or an average daily balance of $-.026$ gm. Subject B in the first kale series, with the same intakes of calcium, showed total outputs of 1.482 and 1.210 gm. for the two successive three-day periods, with corresponding balances of $-.282$ and $-.010$ gm., respectively. The average daily balance was therefore $-.049$ gm. These negative balances indicate that the subjects' minimum requirements of calcium apparently are somewhat more than .40 gm. daily, probably .42 to .45 gm.

As explained above, the first series of spinach diets contained lower amounts of calcium, owing to the unexpectedly low calcium contents of the spinach available at that time. Subject A, on an average intake of 1.015 gm. calcium for each three-day period, excreted 1.335 and 1.269 gm. for the two periods, respectively, giving balances of $-.320$ and $-.254$ gm., or an average daily balance of $-.096$ gm. Subject B, with the same intake of 1.015 gm. calcium for each three-day period, excreted 1.401 and 1.416 gm., respectively,

with balances of $-.386$ and $-.401$ gm. The average daily loss was therefore $-.131$ gm.

When the spinach contained larger amounts of calcium, so that the total intake of calcium was 1.335 gm. for each three-day period, Subject A excreted 1.683 and 1.883 gm. in the two successive experimental periods, with resulting calcium balances of $-.348$ and $-.548$ gm., respectively, with an average daily loss of $-.149$ gm. Subject B excreted 1.583 and 2.068 gm., respectively, during the same three-day periods. The calcium balances were therefore $-.248$ and $-.733$ gm., respectively, averaging $-.164$ gm. per day.

TABLE 2
*Intake and Output of Calcium on Diets With Kale or Spinach
as Chief Source of Calcium*

Subject	Diet	Calcium intake— 3 days	Calcium output—3 days			Balance— 3 days	Average daily balance
			Urine	Feces	Total		
		gm.	gm.	gm.	gm.	gm.	gm.
A.....	Kale	1.200	0.393	0.981	1.374	-0.174	-0.026
A.....	Kale	1.200	0.423	0.755	1.178	$+0.022$	
B.....	Kale	1.200	0.106	1.376	1.482	-0.282	-0.049
B.....	Kale	1.200	0.193	1.017	1.210	-0.010	
A.....	Spinach	1.015	0.153	1.181	1.335	-0.320	-0.096
A.....	Spinach	1.015	0.138	1.132	1.269	-0.254	
B.....	Spinach	1.015	0.024	1.377	1.401	-0.386	-0.131
B.....	Spinach	1.015	0.041	1.375	1.416	-0.401	
A.....	Spinach	1.335	0.133	1.550	1.683	-0.348	-0.149
A.....	Spinach	1.335	0.156	1.727	1.883	-0.548	
B.....	Spinach	1.335	0.130	1.453	1.583	-0.248	-0.164
B.....	Spinach	1.335	0.153	1.915	2.068	-0.733	

In comparing the kale series with the second spinach series, it is interesting to note that with an intake of calcium during the spinach period 11 per cent greater than that of the kale period, the losses of calcium were three to six times greater when spinach was the chief source of calcium than when kale provided most of the calcium. This greater loss on a larger intake shows without doubt that the calcium of the spinach is not so well utilized as that of the kale. These results could not have been due to the effect of fiber, as the fiber contents of the two diets were approximately equal. In comparing the two spinach series, an interesting finding was that when spinach furnished larger amounts of calcium, the losses of calcium from the body were even greater than they were on the smaller intakes of calcium. In the second spinach series, when the calcium intake was

.320 gm. more for each three-day period than in the first spinach series, the losses of calcium from the body averaged .043 gm. per day greater. These data indicate clearly that the calcium of the spinach is not so well utilized as that of the kale. This fact is shown also by comparison of "calcium utilization factors," i.e., ratios of the intakes of calcium to outputs. For the kale this ratio was .94 and .89 for Subjects A and B, respectively. In the first spinach series the ratio had the value of .78 for Subject A and .72 for Subject B; and in the second spinach series, .75 for Subject A and .73 for Subject B. Here again is further evidence that the calcium of kale is more efficiently utilized than that of spinach. In previous work with laboratory animals, Fincke and Sherman (1935), this lower availability of calcium was found to be due mainly, if not entirely, to the presence of oxalate in the spinach. In that investigation the diets were more drastic and were continued for a longer period of time with young, rapidly growing animals. For these reasons the differences were more marked than in the present investigation, which was carried out on adults and therefore involved maintenance of body stores of calcium but not growth. In either case, however, spinach was not a dependable source of calcium.

SUMMARY

In calcium metabolic studies on two healthy women, on diets in which 80 to 86 per cent of the calcium was provided by either kale or spinach, the calcium of the kale was considerably better utilized than that of the spinach. Judging from the results of previous work with rats, this poor availability of the calcium of spinach is evidently largely due to the presence of oxalate in the spinach.

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DEVELOPMENT OF PINK COLOR IN SAUERKRAUT ¹

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Abnormal changes in color and flavor of sauerkraut are some of the most difficult problems with which the manufacturer of this product has to contend. One of these changes, which is most frequent, is the development of pink to red-colored spots or areas throughout the kraut but especially on or near the surface of vats. Such areas may extend from top to bottom of a vat, but ordinarily in such cases the color is far more intense near the surface.

The development of pink areas in kraut have been noted by Butjagen (1904), Wehmer (1905), and Henneberg (1916) and intensively studied by Brunkow, Peterson, and Fred (1921) and Fred and Peterson (1922). These observations, as well as many others, have seemed to be somewhat conflicting in regard to the cause of this defect.

OBSERVATIONS

Pink color has been repeatedly observed by the authors not only in experimental lots but in canned as well as raw kraut. The color has varied from an intense red to pink. The red is common, particularly on the surface of vats of kraut which are covered with a thin layer of salt to inhibit attack by spoilage organisms and insects. Usually such kraut has not been completely fermented before the salt seal is made. The color extends into the product for an inch or so and causes very little loss, possibly less than would result if it were not covered in this manner.

The pink color within the vats of kraut has most often occurred in the areas in which freshly cut cabbage has been dumped during filling of the vats or around the edge of the vat. In some plants salt is added to the cabbage before it is dumped into the vat and may be shaken out in clumps and thereby become concentrated in spots. Occasionally small spots have been noted throughout the vat which varied in color from a light pink to a dirty brown.

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TABLE 1

Chemical Analyses of Samples of Pink Sauerkraut

No.	Total acid as lactic acid	Alcohol as ethyl alcohol	Volatile acid as acetic acid	Non-volatile acid as lactic acid	Ratio volatile to non-volatile acid	Salt	Color	Quality
	pct.	pct.	pct.	pct.				
174	1.29	0.62	.14	1.07	.13	4.3	Pink	Fair
175	1.43	0.17	.14	1.21	.12	2.6	Pink	Fair
176	1.41	0.15	.14	1.20	.12	2.9	Pink	Fair
177	1.33	0.13	.13	1.13	.12	3.0	Pink	Fair
134	1.36	1.33	.27	0.96	.28	3.3	Pink	Unfit
136	1.39	1.08	.30	0.94	.32	4.9	Pink	Poor
100	1.20	1.13	.12	1.02	.12	3.7	Pink	Poor
32	1.1812	1.00	.12	Pink	Unfit
1	1.41	0.68	.32	0.93	.34	2.5	Pink-brown	Poor, tank dry
28	1.38	0.14	.20	1.07	.19	2.8	Pink	Unfit, canned
29	1.23	0.11	.18	0.96	.18	2.3	Pink	Unfit, canned
61	1.2317	0.97	.18	2.4	Pink	Fair, canned
77	1.28	0.25	.17	1.02	.17	1.6	Pink	Fair, canned
66	1.30	0.15	.20	0.99	.20	2.4	Pink	Fair, canned
79	1.40	0.13	.18	1.14	.15	2.0	Pink	Good, canned
101	0.98	0.21	.14	0.78	.18	1.6	Pink	Fair, canned
102	0.98	0.21	.14	0.77	.19	1.6	Pink	Fair, canned
PG1	2.0135	1.49	.23	2.2	Brown	Poor
PG2	2.0741	1.46	.27	2.2	Brown	Poor
G1	1.63	1.30	.31	1.17	.26	2.1	Brown	Fair
G2	1.53	0.50	.28	1.10	.26	2.6	Brown	Fair
G3	2.07	0.64	.51	1.31	.39	1.8	Brown	Fair
H1	1.7630	1.31	.23	2.8	Pink	Poor
H1	1.46	3.2	Pink	Fair
B1	1.4129	0.97	.30	2.2	Brown	Poor
B1	1.5028	1.08	.28	2.2	Brown	Poor
HE1	1.3023	0.96	.24	4.4	Pink	Poor
45	1.6933	1.19	.28	2.5	Good	Excellent
71	1.74	0.19	.31	1.28	.24	2.4	Good	Excellent

Numerous microbiological and chemical analyses of such krauts have been made from time to time. These have always shown the presence of yeasts, rarely in relatively low numbers. Brunkow, Peterson, and Fred (1921) noted the presence of large numbers of yeast cells in red kraut and later Fred and Peterson (1922) isolated three types of yeast capable of causing the development of pink color.

Chemical analyses of such material have shown that usually the volatile acid content is low and the salt content is high, 2.6 to 4.9 per cent (Table 1). Analyses of canned kraut do not always show these facts since the product is diluted in canning. One sample of brilliant red kraut showed the presence of 9.86 per cent salt. According to the definition formulated in a regulation under the Federal Foods and Drugs Act, sauerkraut should be prepared in the presence of not less than two per cent nor more than three per cent of salt. From the data presented and from repeated observations there is considerable chance of pink color developing in kraut containing more than 2.5 per cent salt.

Pink kraut should not be confused with the dark or brown kraut described by Peterson and Fred (1923) which develops after the vats are opened and repacked in barrels. Brown kraut is not associated with raised salt nor does it usually show a low volatile acid content (Table 1). The reverse is more likely to be true. Furthermore, browning usually develops shortly after opening a vat and cannot be removed by washing. It is true that kraut may often develop the pink color after opening of the vat, but in the majority of cases observed the color is present, at least to some extent, when first opened.

DISCUSSION

In the light of present knowledge of the kraut fermentation some of the conditions causing pink colors do not seem to be so entirely unrelated. It has been noted that the salt content of kraut in which pink colors have developed is usually above 2.5 per cent. One might assume that the raised salt content favors the development of yeasts causing this change. In confirmation of previous work by Henneberg (1916) and Brunkow, Peterson, and Fred (1921), however, it has been noted that the pink color may develop in kraut inoculated with a pure culture of a lactic acid bacterium, or in kraut to which a small amount of lactic acid has been added previous to fermentation, the salt content being kept below 2.5 per cent. It has also been observed in kraut to which old kraut juice has been added at the start of the fermentation, on the edge of vats which were not thoroughly cleaned before filling, and in kraut which has been fermented at a relatively high temperature. The latter condition was also noted

by Marten, Peterson, Fred, and Vaughn (1929). Furthermore, Peterson, Parmele, and Fred (1927) have shown the possibility that a low nitrogen in cabbage favors the development of pink kraut. The one of their experimental krauts which turned pink had by far the lowest nitrogen content, .13 per cent. Wadleigh (1932) noted the development of pink kraut from cabbage grown on a nitrogen-starved soil. Fred and Peterson (1922) also noted the necessity of oxygen for color development as well as the favorable effect on color production of certain iron salts, and further noted that color was more intense with xylose as a source of sugar.

A consideration of the normal kraut fermentation may throw light on these apparently unrelated factors which have seemingly influenced the development of pink kraut. It has been shown by Pederson (1930) that in a normal kraut fermentation the various gram-negative types of bacteria are quickly crowded out by growth of bacteria of the species *Leuconostoc mesenteroides*. These, on the other hand, soon reach the limit of acid development and are overgrown by the bacteria belonging to the species *Lactobacillus plantarum* and *Lactobacillus pentoaceticus*, which complete the fermentation. When these types of bacteria develop in proper sequence, a good kraut ordinarily results. On the other hand, this sequence may be influenced by many things. The optimum temperature for the first type of bacterium is considerably lower than for the second and third types of microorganisms, and therefore if the temperature is raised, that is to about 23.9°C. (75°F.), the latter type are favored over the former. The non-gas-producing organisms, *Lactobacillus plantarum*, may predominate in the fermentation, resulting in a lowering of the volatile acid, carbon dioxide, and alcohol content. This same condition can be brought about by inoculation of the fresh cabbage with a culture of *Lactobacillus plantarum* or any other high acid-producing rod that will grow in cabbage. Also since salt and acid are more inhibitory toward *Leuconostoc mesenteroides* than toward *Lactobacillus plantarum*, raising the salt or acid content also has the same effect as raising the temperature. From this, it can be seen that raising the temperature, the salt or acid content and inoculation with certain pure cultures all have the effect of favoring the growth of rod forms over the coccus forms.

Without doubt an important factor which inhibits the development of pink yeast in a normal kraut fermentation is the replacement of air by carbon dioxide as well as the production of acid and alcohol brought about by the early fermentation of *Leuconostoc mesenteroides*. Anything which tends to inhibit the growth of this organism thereby favors the development of pink yeast, whether it be a raised

temperature, raised salt or acid content, or the growth of a high acid-producing organism. It would also seem that a low nitrogen content in the cabbage has the same effect, since it is a well-known fact that the lactic acid-producing organisms are very fastidious in their nitrogen requirements while yeasts will grow with almost any source of nitrogen. In the light of these facts, it is possible that cabbage with a low nitrogen supply does not furnish sufficient available nitrogen for a rapid development of lactic acid bacteria.

CONCLUSION

The development of pink to red color in kraut is due to the growth of certain types of yeasts. The growth of these yeasts is favored by any factor which tends to inhibit or retard the normal sequence of bacterial growth whether it be raised temperature of fermentation, a higher salt or acid content than normal in the cabbage, predominance of certain types of bacteria over others, or a lack of certain nutritives for proper growth.

Such kraut usually shows a lowered volatile acid content, a result of a disturbance of the natural sequence of growth of the bacteria involved in a normal kraut fermentation.

The salt content of pink kraut is often above normal. Salt is usually the inhibiting factor to a natural bacterial fermentation because it is the factor which is most easily changed, but other factors may have the same effect.

Since salting above 2.5 per cent is often an essential factor in causing the development of pink kraut and since packers in general try to pack a product that will comply with regulations, it would seem advisable to change the legal definition for sauerkraut to comply with more desirable conditions of fermentation, that is, to have the upper limits for salt at 2.5 per cent.

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A STUDY OF RATE OF DECOMPOSITION OF HADDOCK MUSCLE AT VARIOUS TEMPERATURES AS INDICATED BY AMMONIA CONTENT^{1,2}

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According to Pierce (1934) the first important commercial use of haddock in the United States dates from about 1870 when smoked fillets were first marketed as "finnan haddie." From that product the public readily turned to the use of fresh haddock with such enthusiasm that for the peak year, 1929, official statistics of the Bureau of Fisheries (1935) show total landings of 261,653,000 pounds with a value of \$9,142,000 to the fishermen of the United States alone for that one year. It is interesting to note that this increase in the use of haddock has paralleled very closely the development of modern refrigeration. In the late nineteenth century the fisherman attempted to rush his catch to some small seacoast town in order that he might dispose of it before spoilage set in. Now, by mechanical refrigeration, the time between the catching and consumption of fish may be extended to several days or, by the even more modern methods of freezing, to several months. It has been a slow process, however, to educate the public to a point where it is willing to believe that a package of frozen fish, caught some months before, may be superior to that which a local merchant may have had in his show case for only a day or two.

This erroneous view may have been strengthened by such statements as that of Vulté and Vanderbilt (1920), "Fish which has been frozen, however, deteriorates rapidly when thawed and decomposition of a very undesirable nature sets in quickly. For this reason, fish should be eaten as fresh as possible." While this statement may be true in substance it is necessary for us to consider with some care just how "quickly" the decomposition sets in and perhaps study means of controlling it.

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As Reay (1935) has pointed out, there are three major methods available for estimating the state of preservation of fish muscle: (a) organoleptic, (b) bacteriological, and (c) chemical. The fish merchant or consumer must depend largely on the first method; for more accurate study one of the other two must be used.

In this connection we have the work of Stewart (1935) in which the rate of bacterial multiplication was followed. Tressler (1920) studied the relationship between amino acids and the condition of fish muscle. Almy (1927) followed the decomposition of fish flesh by means of the hydrogen sulphide formed, while Tillmans and Otto (1924) and Lucke and Geidel (1935) approached the subject from the standpoint of ammonia nitrogen liberated. More recently we have the work of Stansby and Lemon (1933) based on electro-metric titration of the material to determine the buffering effect of the protein molecule before and during decomposition. Stansby (1935) published an additional method for testing the quality of fish tissue based on the condition of the oil which it contained. Beatty and Gibbons (1937) suggested the trimethylamine value of fish as a measure of its freshness.

In this present work an attempt has been made (1) to follow the decomposition of the protein of fish muscle by means of the ammonia nitrogen formed; (2) to compare the rates of decomposition of the fish protein when held at different temperatures; and (3) to compare the rates of decomposition of fish proteins, fresh and after having been frozen by various methods and then defrosted.

RATE OF DECOMPOSITION OF FISH MUSCLE AT VARIOUS TEMPERATURES

During a study of the chemical composition of muscle of common haddock (*Melanogrammus aeglefinus*) it was noticed that there was a striking uniformity in the ammonia nitrogen present in fresh samples. From a number of preliminary analyses made on samples which had been held at various temperatures, it was evident that the storage temperature materially affected the rate of decomposition. In order to compare more accurately the rates of decomposition at various, but controlled, temperatures the following experiment was conducted. A sample of fish muscle, free of bones and skin, was finely ground in a hand meat chopper, mixed, and divided into four parts. Each part was stored in a glass-stoppered bottle at a different temperature as follows:

A. Held in a frozen condition in the freezing unit of a modern household electric refrigerator.

B. Held in the food compartment of the same refrigerator set to maintain a temperature of 4 to 5°C. (39.2 to 41°F.).

C. Held in the food compartment of an ordinary ice refrigerator which was found to maintain a temperature of about 9 to 10°C. (48.2 to 50°F.).

D. Held in a water bath at 24 to 25°C. (75.2 to 77°F.) to simulate summer conditions.

Immediately after grinding and then at regular intervals as indicated (Table 1 and Fig. 1), the ammonia was determined in a sample from each bottle.

The method used was essentially that outlined by the A.O.A.C. Methods of Analyses (1930) for the determination of ammonia in

TABLE 1
Effect of Storage Temperature on Ammonia Production

Days stored after grinding	Milligrams NH ₃ per 100 grams of sample			
	(A) Frozen	(B) 4 to 5°C.	(C) 9 to 10°C.	(D) 24 to 25°C.
0	8.5	6.8	8.5	6.8
2	8.5	6.8	7.7	89.3 ¹
4	3.4	6.0	231.2
7	5.1	6.0	127.5 ²	324.7 ³
9	6.8	7.7	161.5
14	4.3	26.4
21	89.2 ^{1,4}
28	7.7	82.5

¹ Objectionable odor of decomposition. ² Very objectionable odor of decomposition. ³ Decomposition had progressed to such an extent that sample was discarded after analysis. ⁴ Pronounced "fishy" odor in addition to decomposition.

meat and meat products. A 10-gram sample of the finely ground material was placed in a large test tube and thoroughly mixed with 10 to 15 ml. of ammonia-free distilled water, using a heavy glass rod. To this were then added 1 ml. of a saturated solution of potassium oxalate and 20 ml. of a saturated solution of sodium carbonate. This was then rapidly aerated for four and one-half hours. The ammonia was caught in .5 normal sulphuric acid and the excess acid titrated with .1 normal sodium hydroxide.

It will be noted that in the case of Sample D at 24 to 25°C., decomposition had progressed so far in 48 hours that the sample had an objectionable odor, while the ammonia had increased to 89.3 milligrams per 100 grams of sample. By the seventh day this sample had decomposed to such an extent that it showed an ammonia content of 324.7 milligrams per 100 grams of sample. On the other hand, Sample A, held in the frozen condition, showed no sign of decomposition either in respect to ammonia content or general appearance.

A similar sample stored for a period of several months in a frozen condition also showed no increase in ammonia beyond that of experimental error.

RATE OF DECOMPOSITION IN FRESH AND DEFROSTED SAMPLES

Although previous analyses indicated that there was no great change in the fish proteins owing to freezing, the following experi-

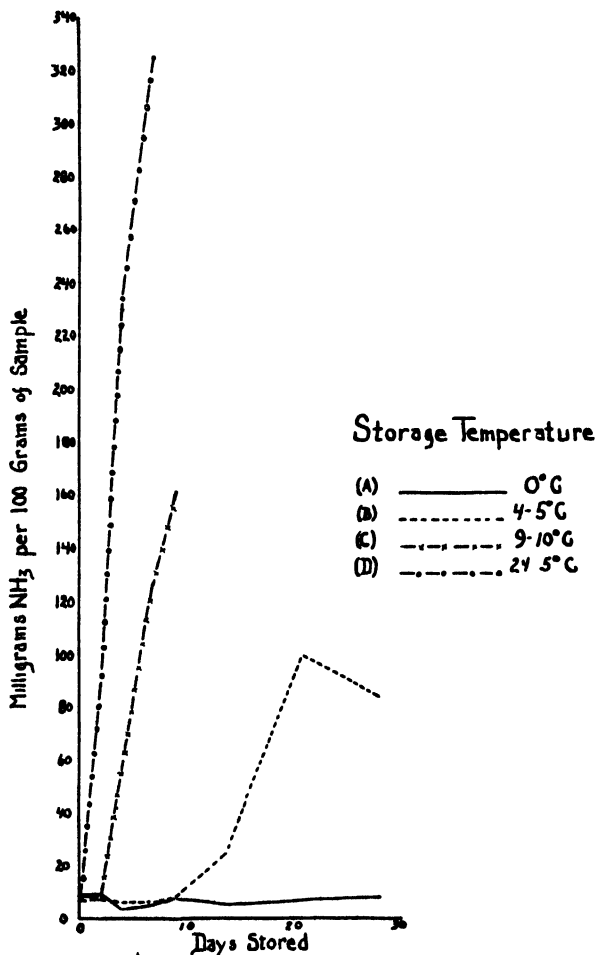


FIG. 1

(A) Ground fish muscle held at 0°C. (B) Ground fish muscle held at 4 to 5°C
(C) Ground fish muscle held at 9 to 10°C (D) Ground fish muscle held at 24 to 25°C

ment was conducted in order to compare more carefully the rate of decomposition, after defrosting, of samples handled according to commercial practice and under identical conditions of storage. The following four samples were compared:

E. Fresh haddock fillets purchased locally. The fish were displayed in an electrically refrigerated show case and filleted as the trade demanded.

F. Commercial fillets frozen by the slow method. This is sometimes called "sharp" freezing.

G. Commercial fillets frozen by the quick or Birdseye method.

H. Fish frozen round by the Birdseye method but otherwise handled in the same manner as the commercial fillets, that is, caught by trawl, gutted at sea as soon as caught, and brought to the Boston Fish Pier in bins with crushed ice in the hold of the vessel.

The fish used in Sample E were claimed by the manager of the store to have arrived that same morning, in ice, by motor truck from Boston about 90 miles away. The ammonia content of this sample compared very closely with other commercial samples purchased

TABLE 2
Ammonia Production in Fresh and Defrosted Fish Muscle

Days stored after grinding	Milligrams NH ₃ per 100 grams of sample			
	(E) Ground fresh fillets	(F) Ground sharp fillets	(G) Ground Birdseye fillets	(H) Ground fish frozen round
0	8.5	8.5	8.5	6.8
1	8.5
3	13.6	12.8
7	59.5 ¹	29.8	6.0
9	89.3 ²	73.1 ³	7.7
11	83.3 ²
14	188.7	167.5	26.4
21	139.4 ⁴	191.7 ⁴	183.7 ⁴	89.2

¹ "Fishy" odor. ² Objectionable odor of decomposition. ³ Stale odor, distinctly different from putrefactive odor. ⁴ Decomposition had progressed to such an extent that sample was discarded after analysis

locally. Samples F, G, and H, after landing at the Boston Pier in ice, were frozen by the methods indicated and held at a temperature of about $-21^{\circ}\text{C}.$ ($-5.8^{\circ}\text{F}.$) for 11 days before grinding. The samples were ground and handled as in the previous experiment except that they were all defrosted and held at a temperature of 4 to $5^{\circ}\text{C}.$ (39.2 to $41^{\circ}\text{F}.$). On the day the samples were ground the ammonia contents were identical for Samples E, F, and G, while that of H was somewhat lower or 8.5 and 6.8 milligrams per 100 grams of sample, respectively. The progress of decomposition in the ground samples was then followed with increases in ammonia (Table 2 and Fig. 2).

In order to check more accurately the rate of decomposition of the ground muscle tissue during the critical period from six to ten days after defrosting, analyses were made (Table 3 and Fig. 3). The

methods and procedure were exactly the same as those used in obtaining the data for Table 2.

Samples B and S were haddock fillets taken from the same catch and frozen at the Boston Fish Pier by the Birdseye and sharp meth-

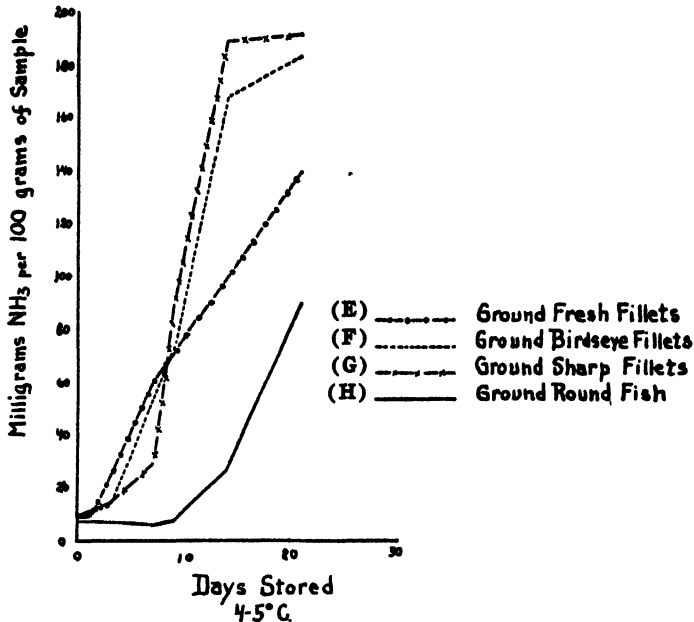


FIG. 2

(E) Ground fresh fillets held at 4 to 5°C. (F) Ground Birdseye frozen fillets held at 4 to 5°C. (G) Ground sharp frozen fillets held at 4 to 5°C. (H) Ground fish, frozen round, held at 4 to 5°C.

ods, respectively. Sample AV was a commercial Birdseye sample purchased from a local retailer after he had held it in stock for some time.

It will be seen (Table 3) that there is practically no ammonia liberated up to six days after defrosting the ground, quick-frozen

TABLE 3
Ammonia Production in Defrosted Fish Muscle

Days stored after grinding	Milligrams NH_3 per 100 grams of sample		
	(B) Ground Birdseye fillet	(AV) Ground Birdseye fillet	(S) Ground sharp fillet
0	7.34	5.72	5.12
6	8.02	8.02
7	5.72	8.02
8	5.63	4.01	12.89
9	17.75	11.26
10	25.77	16.13
11	30.55	17.66	38.66

fillets. From six to 10 days, however, the increase in ammonia formation is both regular and more rapid. It is evident that there is some variation in the rate of decomposition of samples from different catches but that with samples from the same catch similarly

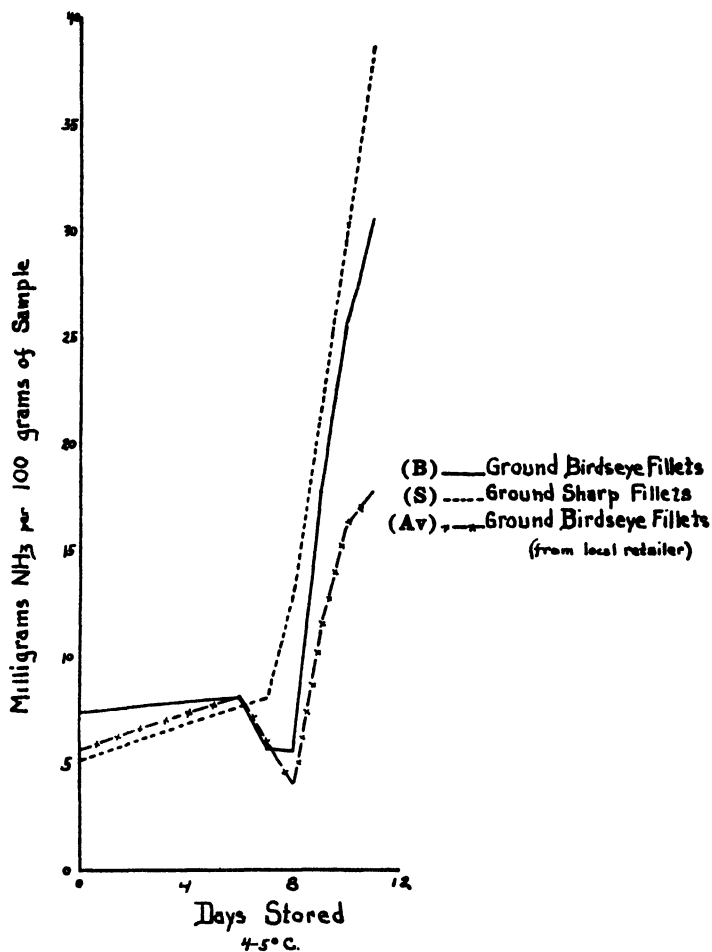


FIG. 3

(B) Ground Birdseye fillets held at 4 to 5°C. (S) Ground sharp frozen fillets held at 4 to 5°C. (Av) Ground Birdseye fillets (purchased locally) held at 4 to 5°C.

processed the rate of decomposition is slower in the samples frozen by the quick or Birdseye method.

DISCUSSION OF RESULTS

As has been pointed out by previous workers, the decomposition of nitrogenous material, whether it be meat or fish, is no simple process. However, since ammonia appears as one of the end products

of protein decomposition, regardless of the intermediate steps, and increases at a fairly regular rate, it seems possible to use it as an index of the rate of decomposition.

In this connection it should be mentioned that there was a very definite correlation between the amount of ammonia present in the sample and the state of preservation as determined organoleptically. With an ammonia content up to 35 milligrams per 100 grams of sample the fish would have been acceptable as human food to the most exacting individual. In most cases, however, when the ammonia content had increased to from 35 to 45 milligrams the freshness of the sample would have been questioned. At 60 milligrams of ammonia per 100 grams of sample there was a stale or "fishy" odor which became a distinctly putrefactive odor as 75 milligrams of ammonia per 100 grams of sample were approached.

The rate of decomposition is materially affected by the temperature at which the ground samples are stored (Table 1 and Fig. 1). The advantage of holding fish at a temperature lower than that obtained by ice refrigeration is clearly shown.

At a given temperature there is little significant increase in the rate of decomposition of ground, defrosted fish when compared with a similar sample which has not been frozen (Table 2 and Fig. 2). Thus, although the consumer may not have the facilities in his home for holding fish in the frozen condition after purchase from the retailer, he is still as well off, so far as actual spoilage is concerned, as if he were to hold fresh fish under the same conditions. Even more can be said in favor of the frozen product before delivery to the consumer. As Lumley (1936) has pointed out, crushed ice is an excellent medium for keeping fish fresh during short periods, such as during shipment from the fishing grounds to a seacoast town; but since the maximum time that fish can be held in ice and be considered really fresh is probably from 12 to 14 days, the distance that it can be shipped inland is extremely limited. This is especially true in view of the present conditions encountered in the haddock industry. At one time good fishing could be found relatively near the New England shore. The boats left early in the morning and were often back with the catch the same day. Now during much of the year it is necessary to fish in more distant waters. The boats are often out of port from eight to 12 days. Thus the time left to get the fish from the coast to an inland consumer is even more limited than formerly. If, however, first-quality fish is filleted and frozen as soon as the boat arrives in port, as is now done, the time available for shipment to distant points becomes almost unlimited.

SUMMARY

The rate of decomposition of ground fish muscle was satisfactorily measured by using the ammonia produced as an index of the protein decomposed. Haddock flesh containing 35 milligrams or less per 100 grams of muscle is normally sound and of good quality.

The rate of decomposition was considerably retarded by lowering the temperature at which the samples were stored, and decomposition is apparently completely checked by holding them in a frozen condition.

Ground flesh of fresh fillets and commercial fillets which had been frozen by the sharp and Birdseye methods and then defrosted at 4 to 5°C. (39.2 to 41°F.) decomposed at practically the same rate. Fish frozen round and then defrosted at the same temperature decomposed more slowly.

ACKNOWLEDGMENT

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STORAGE OF SHELL EGGS¹

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As is the case with all products of agriculture, the production of eggs is seasonal and the consumption comparatively steady, hence the necessity for storage. Unfortunately the usual methods for the storage of foodstuffs are decidedly limited in their application to whole eggs. Obviously neither desiccation nor low-temperature storage can be resorted to. Shell eggs cannot be dried and must not be frozen; therefore, their storage is attended by gradual and recognized deterioration in quality.

For culinary purposes the quality of an egg depends on its appearance when broken out, the ability of the white to whip properly, and, above all, on the taste. Deterioration of egg quality may be judged by a number of well-known signs. Among these are loss of water from the egg, the proportion of thick to thin white, and the apparent viscosity of the thick white remaining. The candling of eggs furnishes a ready but inaccurate method of observing these criteria of spoilage without breaking the shell. Unfortunately none of these criteria lend themselves to anything approaching an accurate measurement, so the judgment of eggs still remains largely a subjective operation. The visible signs of deterioration are known to be accompanied by certain chemical changes in the egg itself. The most studied of such changes are loss of water, change of thick to thin white, change in pH, and loss of carbon dioxide.

Superficially these alterations appear to be related to egg spoilage, and this has led to many trials of the keeping quality of eggs under conditions designed to inhibit one or more of the changes. When successful such inhibition does help to maintain the quality of stored eggs, which seems to indicate that the chemical changes and quality deterioration are in fact related.

Loss of moisture from shell eggs is frequently prevented by increasing the humidity of the storage atmosphere. The increased keeping quality thereby attained is not inconsiderable but is, nevertheless, far from satisfactory. Primarily the picture before the candle, rather than the edible quality of the egg, is improved by keeping the air cell small.

¹ Food Research Division Contribution No. 367.

The change of thick white to thin white is one of the most commonly accepted signs of deterioration in egg quality although it does not apply to the foaming or whipping capacity of the white. The change is apparently due to an alteration in the mucin of the thick white, with the result that some of the viscous mucin disappears and much entrapped thin white is also liberated. The alteration undoubtedly involves the degree to which the proteins are hydrated and is therefore affected by changes in pH, according to Sharp (1929). Further changes in hydration are thought by the author to be due to proteolysis, Balls and Swenson (1934), though this view has been contested by Van Manen and Rimington (1935). Inhibition of the change of thick to thin white appears to be a result of methods designed primarily to slow down other changes, namely, the loss of CO_2 and the increasing alkalinity.

Egg white, as soon as it is removed from the shell, loses carbon dioxide rapidly and becomes alkaline. The source of the gas is most probably the large supply of carbonates present, but its rapid evolution presents still unexplained factors. Not infrequently, the broken-out white of a fresh egg will have attained a pH of 8.9 in two or three hours and will also have given off considerable ammonia by this time. A loss of CO_2 also takes place, though slowly, from eggs in the shell. This holds for temperatures as low as may be employed, in storage, and at such levels has obviously no connection with viability.

The change in pH and the loss of CO_2 are reciprocal; Sharp and Stewart (1931) have shown that both may be inhibited by increasing the concentration of carbon dioxide in the storage space. The egg behaves as though a chemical equilibrium existed and the reaction could be inhibited by the accumulation of one of the end products.

Another method of lessening the loss of CO_2 has been based on decreasing the permeability of the shell to this gas. For commercial purposes it seems impractical to use as a coating any substance that might materially change the appearance of the egg. Therefore, the use of heavy paraffin oils has been advocated by Swenson, Slocum, and James (1932) because they permeate the shell and at least partially stop up the pores without being obvious to the average consumer. One disadvantage of an oiled egg is the tendency of the white to cling to the shell after boiling.

VACUUM- CO_2 -OIL PROCESS

The Bureau of Chemistry and Soils of the U. S. Department of Agriculture has, for several years, conducted experiments with the object of combining removal of air from the egg and decrease in

permeability of the shell into one process for inhibiting egg deterioration. By this means an accumulation of CO_2 seems to be produced in the egg during subsequent storage. A method has been developed by which most of the air is removed from the egg and the shell permeability reduced by the application of oil under pressure. This is accomplished by immersing the eggs in a paraffin oil and evacuating the system, thus removing most of the air borne by the eggs. They are then raised in the vacuum chamber above the surface of the oil and allowed to drain in this position. While some of the oil still adheres to the surfaces of the eggs, the pressure is brought back to about one atmosphere by the rapid introduction of carbon dioxide. The increase in pressure pushes the still-adhering oil into the pores of the shell, at the same time impregnating the oil with carbon dioxide, according to the findings of Swenson and James (1933). The advantages of removing as much oxygen as possible

TABLE 1
Amount of Oil in Shells and Membranes of Processed Eggs

Treatment	Shells	Membranes	Number of determinations
	<i>pct.</i>	<i>pct.</i>	
Unooled.....	.1	3-4	5
Oiled at constant (atmospheric) pressure.....	.3-.6	4-6	5
Oiled with increased pressure.....	.5-.8	16-20	5

from the egg follow from the considerations just outlined. The advantages attending the use of pressure were seen from the following experiment:

When an egg was immersed in oil containing Sudan III, the outside of the shell was stained red, but the inner surface showed merely a number of red patches that indicated an uneven penetration of the oil. When the egg was oiled under reduced pressure, however, it was observed that a later increase in pressure caused very complete penetration of the oil through the shell, producing an evenly and deeply stained inner surface. The stain, however, did not pass the shell membrane. Further experiments showed that the shells of such eggs contained much more oil than those treated at a uniform pressure. Exhaustive extraction with hot ether gave the values for the oil content shown (Table 1).

While the impregnation of the shell with oil is greater where it takes place under increasing pressure, the quantity of oil left on the surface of the shell is less. This is an advantage of the vacuum process, because the surface oil is of little use, being absorbed by the

packing material. It is evident that any excess of oil must be allowed to drain from the eggs before the vacuum is released, and therefore the fluidity of the oil becomes an important detail in carrying out the process on a large scale. Oils having a pour point of 1.7 to 4.4°C. (35 to 40° F.), i.e., well above the temperature of processing and subsequent storage, have given the best results.

COMPARISON OF PROCESSED AND UNPROCESSED EGGS

The reduction in permeability attained by the oiling treatment definitely slows down the rate at which the previously described changes occur in stored eggs.

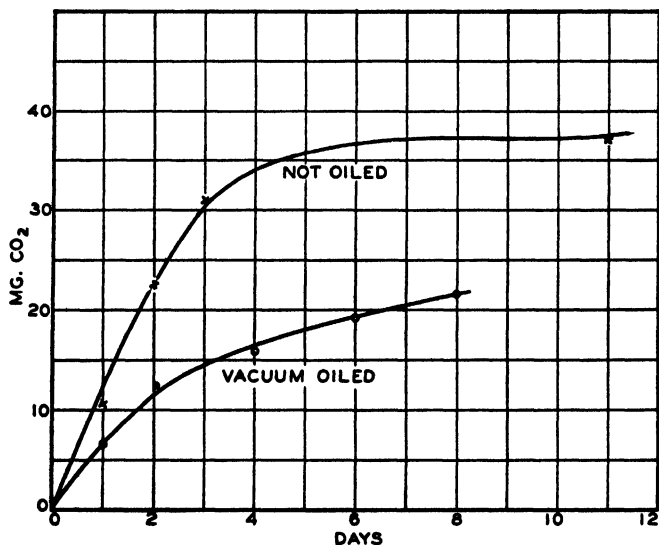


Fig. 1. CO₂ evolution by untreated and treated eggs.

The rate of CO₂ evolution for untreated and treated eggs is shown (Fig. 1) by curves made from averaged results of three runs on single eggs. The eggs were placed in a container set up in a commercial egg-storage room. Air freed from CO₂ was passed slowly through the container and then through standard alkali, which was later titrated.

The change of thick to thin white and the increase in alkalinity on a long storage experiment are shown (Figs. 2 and 3). Two cases of eggs—one untreated, the other vacuum-oiled—were kept in storage at -1.1°C. (30°F.) ($\pm 1^\circ$) and 87 per cent (± 2 per cent) relative humidity. At intervals three eggs were removed from each lot and used for the necessary measurements. The values plotted are arithmetical averages of the three determinations. Because of the

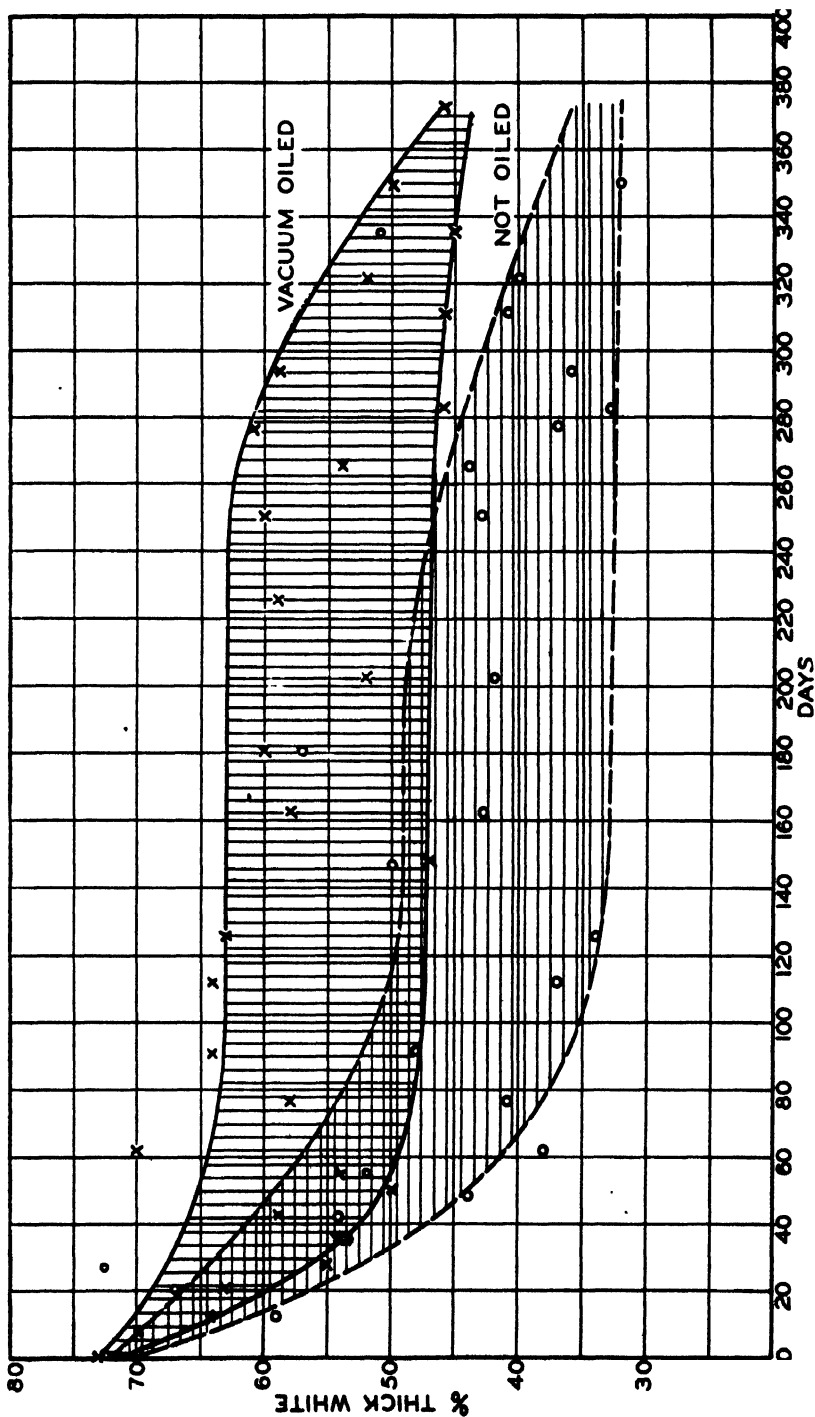


Fig. 2. Decrease in the percentage of thick white of eggs during storage. Oiled eggs, x; untreated eggs, o. Separation of thick from thin white was made by the screen method of Holst and Almquist (1931).

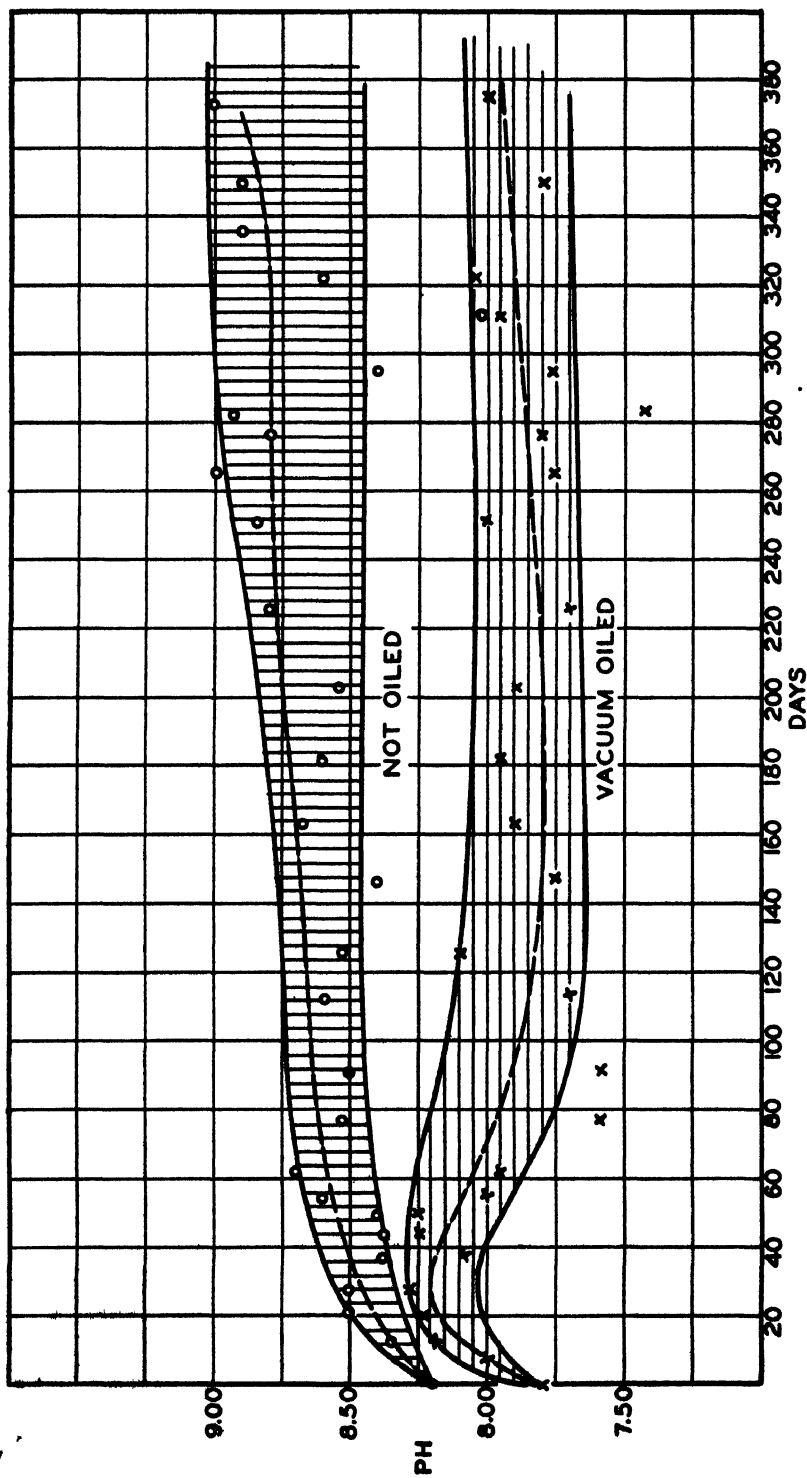


Fig. 3. Change in pH of the white of eggs during storage. Oiled eggs, x; untreated eggs, o.

great variation in keeping quality of individual eggs, the results cannot be expected to show much concordance. The shaded areas (Figs. 2 and 3) are intended to denote the probable range of individual results. It is evident that the two types of eggs exhibit different trends, the oiled eggs changing in each instance less than the controls.

DETAILS OF A LARGE-SCALE EXPERIMENT

A trial of the vacuum-oiling process was finally made on a semi-plant scale and under typical industrial conditions. Eight hundred cases of eggs were collected during a single week at Omaha, Nebraska, given a preliminary candling to insure good initial quality in the experimental material, and mixed together in order to avoid a concentration of eggs from any particular locality. The eggs were then subdivided into four lots of 200 cases each. These lots were treated as follows:

A—No treatment (controls).

B—Oiled in an open immersion-type commercial oiling machine, using plain paraffin oil.

C—Oiled in the same machine as B, but with oil continuously charged with carbon dioxide. The oil contained about 30 per cent CO_2 by volume during its use.

D—Oiled by the vacuum- CO_2 method. The eggs were immersed in oil and the pressure in the chamber reduced to 12 inches of mercury (an 18-inch vacuum). This required 1.5 to 2 minutes. The eggs were then raised above the surface of the oil and CO_2 admitted into the vacuum chamber until atmospheric pressure was reached.

The same oil at the same temperature of $51.7^\circ\text{C}.$ ($125^\circ\text{F}.$) was used on each lot of eggs. Each of the four lots was next halved, one-half of each lot being put into commercial storage in Omaha, while the other half was shipped by railroad to New York and put into similar storage there. Storage at $-1.1^\circ\text{C}.$ ($30^\circ\text{F}.$) ($\pm 1^\circ$) and 87 per cent (± 2 per cent) relative humidity lasted six months.

At four stages of the experiment—(a) before treatment, (b) after treatment and before shipment, (c) after shipment and before storage, and (d) after final storage—every tenth case was opened and all the eggs therein graded before the candle. The same cases, however, were not graded twice. The grading was done by two licensed egg-graders, Dr. Robert Mericle and Mr. Lester Kilpatrick, Associate Marketing Specialists of the Bureau of Agricultural Economics, whose results are summarized here (Table 2).

It is evident from the results of this test that decided protection of the quality is obtained by any of the oiling treatments during straight storage and during transportation. Transportation, however, affects the subsequent keeping qualities of eggs most adversely,

TABLE 2

Summary of Results in Large-Scale Experiment

Eggs Stored Six Months at Omaha (400 Cases ¹)											
Treatment	Number of cases treated	Number of cases graded	Initial grading ²				Final grading ²				Eggs remaining in the two top grades
			I	II	III	IV	I	II	III	IV	
Grade.....									pct.
Untreated.....	100	10	31.7	65.8	0.8	0.4	0	57.5	38.3	4.2	59.0
Dipped in oil.....	100	10	32.9	63.1	1.7	0.1	4.6	72.5	20.8	2.1	80.3
Dipped in CO ₂ oil.....	100	10	28.5 ³	68.1	1.4	0.1	4.0	73.7	15.5	1.8	80.4
CO ₂ -oil-vacuum treatment.....	100	10	49.1 ³	49.5	0.2	0.0	6.8	76.7	12.6	1.8	84.6

Eggs Treated in Omaha, Immediately Shipped to New York (Transit Time, Three Days), and Stored for Six Months																
Treatment	Number of cases ¹ treated	Number of cases ¹ graded	Grading on departure from Omaha ²				Grading on arrival in New York ²				Eggs remaining in the two top grades during transit	Grading after storage ²	Eggs remaining in the two top grades after transit and storage	pct.		
			I	II	III	IV	I	II	III	IV						
Grade.....														
Untreated.....	100	10	34.3	64.4	0.5	0.2	1.5	76.1	19.2	1.6	78.3	0	9.6	80.9	7.2	9.7
Dipped in oil.....	100	10	36.6	61.7	0.6	0.1	13.4	72.6	11.0	1.1	87.5	0	39.4	53.5	4.1	40.1
Dipped in CO ₂ oil.....	100	10	53.4 ³	44.1	0.5	0.2	16.9	61.9	15.8	1.7	80.8	0	35.2	45.0	4.4	36.2
CO ₂ -oil-vacuum treatment..	100	10	37.9 ³	60.6	0.4	0	17.1	65.4	13.9	1.7	83.8	2.2	63.4	32.0	2.3	66.6

¹ A case contains 360 eggs. ² Expressed as per cent in each grade. Grade I denotes U. S. Specials; II, U. S. Extras; III, U. S. Standards; IV, U. S. Trades. ³ Almquist, Givens, and Klose (1934) have observed that CO₂ quickly increases the cloudiness of egg albumin and therefore may change the grading results. The variations found on initial grading may be referable to this phenomenon

and it is under these conditions that the vacuum-oiling process shows to greatest advantage. Not 10 per cent of the control eggs starting in the two top grades remained there after transportation and storage. On the other hand, about two-thirds of the vacuum-oiled eggs remained in the two top grades as well as did one-third of the plain oiled eggs.

COST OF OIL TREATMENT

It was not possible while working on this semi-plant scale to determine the cost of treatment accurately. Nevertheless, a rough estimate was made, based on the actual labor and materials employed in these runs. On a large scale the unit cost should be lower. This is especially true because the vacuum-oiling machine was a

TABLE 3
Cost of Oil Treatment

Items of expense	Plain oil dip	Vacuum-CO ₂ oiling
Labor.....	2.8 ¹	7.6
Oil.....	1.7	3.3
Power and overhead.....	1.8	1.7
CO ₂	0.0	2.5
Total.....	6.3	15.1

¹ Figures represent cents per case of 360 eggs.

small one that handled only half a case at a time. The expense of treatment as estimated is itemized (Table 3); no allowance has been made for additional space and time required in the factory.

ACKNOWLEDGMENT

The author wishes to express his gratitude for help and assistance received in the conducting of these experiments. Despite the fact that the work entailed serious inconvenience and perhaps financial loss to the coöperating business houses, their aid and coöperation were always cheerfully forthcoming. In particular, personal thanks are due Dr. Mericle and Mr. Kilpatrick, of the Bureau of Agricultural Economics, who graded the eggs; to Dr. G. F. Stewart of Omaha; and to Mr. J. Redding and Mr. B. Teevem of New York, for untiring assistance, interest, and willingness to place their vast experience of egg-handling at the author's disposal.

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INHIBITORY PROPERTIES OF HORSE-RADISH VAPORS

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Walton, Herbold, and Lindegren (1936) showed that the vapors from crushed garlic exhibited bactericidal properties. More recently Lovell (1937) reported the same property for the vapors from crushed onion. In some preliminary experiments it was found that the vapors from crushed horse-radish likewise inhibit the growth of a variety of organisms.

EXPERIMENTAL WORK

In order that results could be readily compared the same method used by the other workers on this subject was followed. Horse-radish roots purchased on the open market were finely ground in a meat chopper. One-gram amounts of this material were weighed and placed in the tops of inverted Petri plates, the bottoms of which contained approximately 15 c.c. of a suitable medium. Glycerine agar was used for the acid-fast organisms and nutrient agar for the other types. The plates were sealed and incubated at 10, 20, and 37.5°C. (50, 68, and 99.5°F.) for varying lengths of time. After the exposure period the covers of the Petri plates were removed and replaced by sterile tops and immediately streaked with a suspension of the test organism. The plates were then resealed and incubated at 37.5°C. for at least one week. Controls were made in all experiments.

Prior to the exposure period the agar plates were held for several hours at the same temperature at which the exposure was to be made. The organisms used were 24- to 48-hour slant cultures of *Serratia marcescens*, *Bacillus subtilis*, *Escherichia coli*, *Mycobacterium phlei*, and *Mycobacterium tuberculosis* var. *hominis*. Heavy suspensions of these organisms were made in nutrient broth prior to streaking the exposed plates.

A comparative scale was used in estimating the amount of growth, by which 4 indicated full growth as in the controls, 3 indicated from three-quarters to full growth, 2 indicated one-half to three-quarters of full growth, and 1 indicated any growth to one-half of full growth.

RESULTS

In order to determine the effect of different temperatures on the volatility of the inhibitory substance, exposures of eight minutes

TABLE 1
Degree of Growth of Organisms on Agar Plates After Exposure to Horse-Radish Vapors

Organism	Exposed at 10°C. (50°F.)							Exposed at 20°C. (68°F.)							Exposed at 37.5°C. (99.5°F.)							
	360 ¹	180	120	60	32	16	8	0	180	120	60	32	16	8	0	180	120	60	32	16	8	0
<i>Serratia marcescens</i>	0	0	0	1	1	2	2	4	0	0	0	0	3	4	4	0	0	0	0	0	3	4
	0	0	0	1	1	2	2	4	0	0	0	3	3	4	4	0	0	0	0	1	3	4
	0	0	1	1	2	2	2	4	0	0	1	3	4	4	4	0	0	0	2	2	3	4
	0	0	1	1	3	3	3	4	0	0	1	3	4	4	4	0	0	0	3	3	4	4
<i>Bacillus subtilis</i>	0	0	0	0	1	1	2	4	0	0	0	0	1	4	4	0	0	0	0	0	3	4
	0	0	0	1	1	1	2	4	0	0	0	0	1	4	4	0	0	0	0	0	3	4
	0	0	0	1	1	2	2	4	0	0	0	1	4	4	4	0	0	0	0	2	3	4
	0	0	1	1	1	2	3	4	0	0	0	1	4	4	4	0	0	0	0	2	3	4
<i>Escherichia coli</i>	1	1	1	1	1	3	4	4	0	0	1	2	3	4	4	0	0	0	2	3	3	4
	1	1	2	2	2	3	4	4	0	1	1	2	3	4	4	0	0	1	2	3	3	4
	1	2	2	2	3	4	4	4	1	2	3	3	3	4	4	0	0	1	2	3	4	4
	2	2	2	3	3	4	4	4	1	2	3	3	3	4	4	0	0	2	3	3	4	4
<i>Mycobacterium phlei</i>	0	0	0	1	1	1	3	4	0	0	0	1	1	1	4	0	0	0	0	0	1	4
	0	0	0	1	1	2	3	4	0	0	0	1	1	2	4	0	0	0	1	1	3	4
	0	0	1	1	1	2	3	4	0	0	1	1	1	3	4	0	0	0	1	2	3	4
	0	1	1	1	2	3	3	4	0	1	1	2	2	3	4	0	0	1	2	3	3	4
<i>Mycobacterium tuberculosis</i> var. <i>hominis</i>	0	0	0	1	1	3	4	4	0	0	0	0	0	2	4	0	0	0	0	0	3	4
	0	0	0	1	1	4	4	4	0	0	0	1	1	2	4	0	0	0	1	2	4	4
	0	1	1	2	2	4	4	4	0	0	1	1	1	2	4	0	0	0	1	3	4	4
	0	1	2	2	2	4	4	4	0	1	1	1	1	3	4	0	0	0	1	4	4	4

^a The number at the top of each column indicates time of exposure in minutes.

to three hours were made at 20°C.(68°F.) and 37.5°C.(99.5°F.). The exposure period was lengthened to six hours at 10°C.(50°F.). The degree of growth is shown when various temperatures of exposure are used (Table 1).

The inhibitory effects are most pronounced at 37.5°C., as might be expected. The organisms differed in their resistance to the vapors from horse-radish. *Bacillus subtilis* was the least resistant, while *Escherichia coli* was the most resistant. *Serratia marcescens*, *Mycobacterium phlei*, and *Mycobacterium tuberculosis* were almost identical in their resistance to the horse-radish vapors. By computing the averages of the results obtained (Table 1) the effect of the temperature on the volatility of the inhibitory substance could be shown (Table 2).

Experiments were made to ascertain the time required to exhaust the inhibitory substance emitted from the one gram of ground horse-radish. Three-hour exposure periods were used, after which the exposed agar plates were removed and sterile covers put on, streaked, and resealed. Fresh agar plates were placed over the original one-gram amounts of ground horse-radish and the plates resealed and reincubated for three hours more. The same procedure was then repeated. The results (Table 3) indicate that the inhibitory effect of the horse-radish vapors is more rapidly exhausted than is that of garlic but more lasting than the effect of the vapors from onion.

DISCUSSION

A comparison of data where similar test organisms were used indicates that the vapors from crushed horse-radish have a greater inhibitory effect than those from crushed garlic or onion. The inhibitory substance in horse-radish is probably different from that in garlic or onion.

In the reports on the toxic effects of the fumes from crushed garlic and onion *Bacillus subtilis* and *Serratia marcescens* were more resistant than certain of the *Mycobacterium* species. In the experiments reported above *Bacillus subtilis* was less resistant than *Mycobacterium phlei* and *Mycobacterium tuberculosis*. This discrepancy may be due to a difference in the resistance of the species used as test organisms.

CONCLUSIONS

1. The vapors from crushed horse-radish roots have a strong inhibitory effect.
2. The effect of these vapors is most pronounced at 37.5°C. (99.5°F.) and decreases with the lowering of the temperature of exposure.

TABLE 2
Inhibitory Effect of Horse-Radish Vapors at Various Temperatures

Organism	Exposed at	Control	8 min.	16 min.	32 min.	1 hr.	2 hr.	3 hr	6 hr.
<i>Serratia marcescens</i>	37.5°C. (99.5°F.)	4	3.25 ¹	1.75	1.25	0	0	0
	20°C. (68°F.)	4	4.00	3.50	2.25	0.50	0	0
	10°C. (50°F.)	4	2.25	2.25	1.75	1.00	0.50	0	0
<i>Bacillus subtilis</i>	37.5°C. (99.5°F.)	4	3.00	1.00	0	0	0	0
	20°C. (68°F.)	4	4.00	2.50	0.50	0	0	0
	10°C. (50°F.)	4	2.25	1.50	1.00	0.75	0.25	0	0
<i>Escherichia coli</i>	37.5°C. (99.5°F.)	4	3.50	3.00	2.25	1.00	0	0
	20°C. (68°F.)	4	4.00	3.00	2.50	2.00	1.25	0.50
	10°C. (50°F.)	4	4.00	3.50	2.00	2.00	1.75	1.50	1.25
<i>Mycobacterium phlei</i>	37.5°C. (99.5°F.)	4	2.50	1.50	1.00	0.25	0	0
	20°C. (68°F.)	4	2.25	1.25	1.25	0.50	0.25	0
	10°C. (50°F.)	4	3.00	2.00	1.25	1.00	0.50	0.25	0
<i>Mycobacterium tuberculosis</i> var. <i>hominis</i>	37.5°C. (99.5°F.)	4	3.75	2.50	1.00	0.25	0	0
	20°C. (68°F.)	4	2.00	0.75	0.75	0.50	0.25	0
	10°C. (50°F.)	4	4.00	3.75	1.50	1.50	0.75	0.50	0

¹ Each number represents an average of four experiments

3. The inhibitory property of the vapors from horse-radish is greater than that of garlic or onion but is more quickly exhausted than that of garlic when tested under similar conditions.

TABLE 3
*Exhaustion of Vapors From One Gram of Horse-Radish at
37.5°C. (99.5°F.)*

Organism	Control	First 3-hr. period	Second 3-hr. period	Third 3-hr. period
<i>Serratia marcescens</i>	4	0 ¹	3.5	3.5
<i>Bacillus subtilis</i>	4	0	2.5	3.25
<i>Escherichia coli</i>	4	1	4.0	4.0
<i>Mycobacterium phlei</i>	4	0	2.0	3.5
<i>Mycobacterium tuberculosis</i> var. <i>hominis</i>	4	0	2.0	4.0

¹ Each number represents an average of four experiments.

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A NOTE ON YEAST OBTAINED FROM SLIMY SAUSAGE

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(Received for publication, March 5, 1938)

Wiener sausages often become covered with a pasty, yeast-like slime resulting from the growth of microorganisms. According to Tanner (1932), who has summarized the literature on this subject, slime formation on sausage is caused by bacteria. In regard to the possibility of yeast being involved he states, "It has been stated now and then that yeasts were the cause of the spoilage. This is probably due to the appearance of the sausage and not to the data which have been collected in bacteriological examination." Cesari (1919) and Cesari and Guilliermond (1920) reported on several species of yeasts obtained from certain types of dried sausage produced in France. There is no further information available concerning the occurrence of yeast in the slime on fresh sausage.

The writers have examined microscopically the slime obtained from several samples of slimy wiener sausage submitted for observation. All samples of slime examined consisted of a mixture of yeast and various forms of bacteria. Several cultures of yeast, capable of forming a dull slime when inoculated on sterile sausage, were isolated and studied morphologically and physiologically. The methods used were those of Stelling-Dekker (1931), Lodder (1934), and Guilliermond (1928). Ridgway (1912) was followed for color descriptions. A discussion of the characteristics of the yeasts studied is limited to those of a single organism, since all cultures obtained from sausage proved to be similar.

MORPHOLOGY

Cells from 1-, 3-, and 10-day wort cultures and 3-, 10-, and 70-day wort-agar cultures are spherical to globose. Cells from the same culture, range $(2.5-6) \times (2.5-6) \mu$ and average $4 \times 4 \mu$. Vegetative reproduction is by many-sided budding with the subsequent formation of small clusters.¹ Clusters containing more than two cells rarely form in young (18- to 36-hour) cultures growing in 5° Balling liquid wort. Frequently large clusters form, however, when a 15° Balling wort medium is used or when the cultures are allowed to stand for several days before being examined.

Spore formation occurs on cucumber and potato wedges and Garodkowa agar slants after 12 days but does not occur on gypsum

blocks, sausage, washed agar, wort agar, wort gelatin, liquid wort, grape juice or on carrot, beet, or dill pickle wedges in 70 days. An isogamic (dumb-bell like) or heterogamic copulation always precedes spore formation. Adelphogamy² occurs frequently in cultures growing on potato wedges and Garodkowa agar slants.

Each ascus, resulting from the copulation of two cells, contains a single, verrucose, spherical spore having a centrally located oil drop. The spore dimensions range $(2-3.5) \times (2-3.5) \mu$ and average $3 \times 3 \mu$. They do not vary markedly with the temperature of incubation. Ascus dimensions, however, vary considerably with the incubation temperature and thereby affect the ease with which the spores may be observed. Mrak and Bonar (1938) have already discussed these variations in detail. Spore germination is similar to that described by Klöcker (1924) for *Debaryomyces globosus*.

A thin, smooth to slightly irregular, drab gray film forms in 24 hours in liquid wort incubated at 20 to 24°C. (68 to 75.2°F.). Films gradually become thinner and finally disappear upon prolonged storage. Rings also form and then gradually disappear during storage. Considerable sediment forms, in time, at the expense of the film and ring.

Giant colonies on wort gelatin liquefy the gelatin after 30 days of storage at 20°C. (68°F.). Wort-agar giant colonies have a pale olive-buff color, lobate-lobulate edges, smooth to bullate surfaces with dull centers, glistening borders, and umbonate forms. Wort-agar streak cultures have lacerate borders, smooth to finely bullate surfaces, glistening wood-brown color, and slightly convex forms.

PHYSIOLOGY

Dextrose, levulose, mannose, and sucrose are fermented very slowly and maltose doubtfully. Arabinose, xylose, galactose, lactose, raffinose, rhamnose, dextrin, starch, inulin, and glycerine are not fermented. Asparagine, peptone, and glycine are utilized but KNO_3 , $(\text{NH}_4)_2\text{SO}_4$, and urea do not support growth on auxanogram plates. Concentrations of one-half per cent of citric, tartaric, malic, or lactic acids or a concentration of one-fourth per cent of acetic acid in Stelling-Dekker's synthetic medium do not support growth.

The temperature range for growth in liquid wort is 7 to 31°C. (44.6 to 87.8°F.) but the optimum range is 22 to 28°C. (71.6 to 82.4°F.). The temperature range for growth on sausage is 4 to 31°C.

¹ The term cluster is used in place of the German word "sprossverbaende."

² Adelphogamy is used in accordance with the definition of Hartmann (1918) referring to pseudogamy between mother and daughter cells. Above it is used to refer to copulation between mother and daughter cells before separation.

(39.2 to 87.8°F), but heavy growth occurs within the range of 10 to 28°C. (50 to 82.4°F.).

These organisms are unusually tolerant to high concentrations of salt. Growth occurs with film formation in cucumber juice and in 15° Balling wort containing 20 per cent of salt.

TAXONOMY

The yeasts isolated from fresh sausage are cultures of *Debaryomyces* closely resembling *Debaryomyces Guilliermondii* Stelling-Dekker. Lodder (1932) has pointed out that the characteristics given for *D. Guilliermondii* by Stelling-Dekker are not sufficient to distinguish it from *D. membranefaciens* and has used the size of clusters occurring in young liquid wort cultures as an important character for distinguishing the two species. According to Lodder, clusters from young cultures of *D. Guilliermondii* never contain more than two cells, whereas those of *D. membranefaciens* usually contain more than two cells. The writers have observed that cultures of *D. Guilliermondii* obtained from the C.B.S. often develop clusters containing more than two cells when cultured in liquid wort. Cultures examined 18 hours after inoculation in 5 to 8° Balling wort contain very few clusters with more than two cells. When *D. Guilliermondii* is grown in 15° Balling wort, however, or when cultures in 5° Balling wort are stored for 48 hours before observation, large clusters occur more frequently. The yeasts isolated from the fresh sausage slime behave in a similar manner and are believed to be cultures of *D. Guilliermondii*. The characters of *D. Guilliermondii* Dekker and *D. Guilliermondii* var. *nova zeelandicus* Lodder and the cultures isolated from the sausage slimes have been compared in the laboratory. The three organisms are similar in most respects including salt tolerance. However, the fermentation characters of the cultures isolated from sausage and *D. Guilliermondii* var. *nova zeelandicus* are so nearly alike that the organisms are considered to be *D. Guilliermondii* var. *nova zeelandicus* Lodder.

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EFFECT OF COAGULATION ON PRESS FLUID, SHEAR FORCE, MUSCLE-CELL DIAMETER, AND COMPOSITION OF BEEF MUSCLE¹

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Much work has been done on meat in the raw state but until recent years little study has been devoted to the effect of cooking on chemical composition and physical properties of meat, which so greatly determine its nutritive value and palatability. The present study was undertaken to determine (1) the effect of coagulation of the triceps brachii, adductor, and longissimus dorsi muscles of beef on shear force, diameter of muscle fibers, total moisture, and ether extract; (2) the effect of different degrees of coagulation or different internal temperatures of semitendinosus muscle on press fluid, total moisture, total losses, shear force, and diameter of muscle fibers.

EXPERIMENTAL PROCEDURE

All meat was prepared and cooked by the methods recommended by the cooking committee of the Cooperative Meat Investigations, reported by Alexander, Clark, and Howe (1933), and all cooking data were recorded. The longissimus dorsi (11-12th rib), adductor (round), and triceps brachii (bread and butter cut) muscles from the right sides of 13 animals were coagulated to an internal temperature of 58°C.(136°F.) at 150°C.(302°F.). The left cuts were analyzed raw. One of the four comparable roasts from semitendinosus muscle was left raw; the other three were coagulated at 150°C., one to 58°C., one to 67°C.(153°F.), and one to 75°C.(167°F.). The longissimus dorsi, adductor, and triceps brachii muscles were sampled at maximum temperature and the semitendinosus muscle at 40°C.(104°F.).

Fat and bone were removed from the muscle, and it was halved through the thermometer hole perpendicular to the muscle fibers. A slice 1.25 cm. thick was taken, from the center of which two samples were cut parallel to the muscle fibers with a borer 1.25 cm. in diameter. The remainder of the slice, except for one-half inch around the edge, was ground for chemical analyses. Measurement of muscle fibers was made on muscle from the remainder of the half. From the other

¹ Scientific Journal Series paper No. 1598, Minnesota Agricultural Experiment Station.

² Deceased July 10, 1938.

TABLE 1

*Tenderness (Shear Force) and Diameter of Muscle Fibers of Raw and Cooked
Triceps Brachii, Adductor, and Longissimus Dorsi Muscles of Beef*

Series No.	Shear force						Diameter muscle fibers					
	Triceps brachii			Adductor			Longissimus dorsi			Triceps brachii		
	Raw		Cooked	Raw		Cooked	Raw		Cooked	Raw		Cooked
	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	microns	microns	microns
1.....	64.91	48.76	62.94
2.....	14.50	15.20	19.40	21.60	50.45	39.67	51.47
3.....	20.90	20.80	23.60	20.10	19.80	14.20	62.92	50.79	61.58
4.....	20.60	14.20	21.70	24.50	18.30	15.50	62.43	59.63	61.16
5.....	18.60	14.80	19.10	17.30	15.60	10.00	51.73	44.93	54.36
6.....	15.30	8.60	12.90	13.50	12.10	13.00	60.90	56.40	67.95
7.....	25.21	18.81	33.46	26.38	17.21	19.96	67.31	61.51	74.90
8.....	28.04	28.29	35.00	32.71	31.63	22.71	68.05	58.10	74.51
9.....	18.29	21.25	35.50	36.54	25.63	24.55	73.01	58.88	74.90
10.....	17.71	22.59	24.88	33.50	19.83	18.21	71.51	55.10	72.83
11.....	21.83	27.27	25.96	37.21	26.75	17.09	64.98	49.97	77.79
12.....	36.75	26.25	30.09	34.00	26.79	21.71	58.96	57.89	66.44
13.....	20.00	25.21	32.16	24.71	21.08	23.33	75.15	58.14	65.98
Mean.....	21.48	20.27	26.16	26.84	21.34 ¹	18.21	64.02 ²	53.83	66.68 ²
											57.90	64.71 ²
												56.72

¹ Significant difference. ² Very significant difference.

half of the roast two samples for tenderness determinations were cut parallel to the muscle fibers with a borer one inch in diameter.

Press Fluid: Each sample was weighed, wrapped in unsized filter cloth, and pressed for 10 minutes under a pressure of 250 pounds with the pressometer developed by Child and Baldelli (1934). The difference in weight before and after pressing was referred to as press fluid.

Tenderness: Each sample was sheared at the center and at the center of each half by the Minnesota modification of the shear-stress apparatus developed by Child and described by Child and Satorius (1938). The number of pounds of force was recorded on a gauge as shearing bars were pulled across a dull blade with a triangular opening through which the sample of meat was placed.

Diameter of Muscle Fibers: The diameter of muscle fibers¹ was measured with the aid of an eye-piece micrometer as described by Brady (1937). The diameters of 50 fibers were averaged for each sample of meat.

Chemical Analyses: Total moisture was determined on two samples, weighing three to five grams each, which were dried for two hours at 60 to 65°C. (140 to 149°F.) in an air oven and for five hours at 100°C. (212°F.) under a pressure of 25 to 30 millimeters.

Ether extract was determined by the Soxhlet method as described in the official methods of the Association of Official Agricultural Chemists (1930) on the first six animals of the series, but owing to poor checks a second method² was used in which a 10-gram sample was ground, dried, and extracted with petroleum ether. Aliquot portions of the solution were dried at 100°C. (212°F.). The amount of material extracted by the two methods was compared on 12 samples of meat. The "t" test showed no difference in the average amount extracted.

DISCUSSION OF RESULTS

Coagulation did not affect the tenderness of triceps brachii and adductor muscles, but the longissimus dorsi muscle became more tender with coagulation (Table 1). Lowe (1933) found that the longissimus dorsi muscle cooked well-done was less tender than the uncooked when measured both by the penetrometer and the shear-stress apparatus. The apparent discrepancy in results here may be attributed to the difference in stage of coagulation of the cooked muscle. The effect of degree of coagulation will be pointed out later in this

¹ Experimental work was done by D. E. Brady, Animal Husbandry Department, University of Minnesota.

² Courtesy of Swift and Company, Chicago, Illinois.

TABLE 2

Ether Extract (Calculated on Wet Basis) and Total Moisture in Raw and Coagulated Triceps Brachii, Adductor, and Longissimus Dorsi Muscles of Beef

Series No.	Ether extract (wet basis)						Total moisture					
	Triceps brachii		Adductor		Longissimus dorsi		Triceps brachii		Adductor		Longissimus dorsi	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
1.....	pct. 2.34	pct. 4.09	pct. 3.79	pct. 2.85	pct. 3.91	pct. 3.39	pct. 74.17	pct. 69.89	pct. 72.15	pct. 69.21	pct. 72.63	pct. 70.46
2.....	2.07	3.33	1.96	3.45	1.37	3.86	75.26	72.43	73.47	69.90	74.69	71.55
3.....	2.54	3.22	1.52	3.00	2.30	3.61	74.61	75.12	74.46	71.95	73.81	69.85
4.....	4.67	3.71	2.55	2.22	2.20	5.34	73.51	72.28	73.06	72.50	73.16	70.98
5.....	1.63	3.34	1.69	2.89	2.49	4.30	75.17	73.96	75.55	72.88	74.18	73.06
6.....	1.74	4.12	1.62	2.62	2.84	5.82	74.71	71.63	75.14	73.60	75.82	69.15
7.....	3.46	3.81	2.25	2.65	4.91	5.11	72.60	69.85	71.69	70.72	69.63	69.32
8.....	2.09	3.14	1.63	3.18	4.21	4.28	75.89	70.68	74.06	71.35	72.42	69.96
9.....	3.06	4.13	2.42	3.95	5.59	8.95	74.11	71.55	72.73	70.27	73.90	67.47
10.....	3.95	5.65	2.29	3.84	5.52	11.35	74.69	67.62	74.27	69.83	72.09	67.08
11.....	4.80	4.79	2.11	3.44	5.82	6.40	73.71	71.01	74.76	70.09	72.67	70.50
12.....	2.75	4.41	2.63	3.36	4.05	7.17	74.73	70.70	73.87	70.13	73.45	68.87
13.....	5.85	7.65	5.69	7.91	13.29	11.81	73.79	70.42	71.22	63.61	63.76	66.99
Mean.....	3.15 ¹	4.26	2.47 ¹	3.49	4.50 ¹	6.26	74.38 ¹	71.32	73.57 ¹	70.46	72.48 ¹	69.63

¹ Very significant difference.

paper. The diameter of muscle fibers was decreased 12 to 16 per cent during coagulation of the three muscles to 58°C.(136°F.). Since the length of the fiber was not measured, this cannot be considered a true indication of total shrinkage in volume.

About four per cent of the total moisture content was lost upon coagulation of the three muscles (Table 2). With coagulation of the protein, water is released from the colloidal structure, some of which is volatilized, and the cell becomes shrunken. Ether extract was found to be increased from 35 to 40 per cent of the original amount by coagulation (Table 2). These calculations were first made on the wet basis. Since water is lost during coagulation it was thought that the increase in ether extract might be due to concentration of solids. Recalculation of ether extract on the dry basis, however, still showed a significant increase during coagulation. This increase is evidently due to infiltration of melted surface fat. The greatest increase was found in the longissimus dorsi muscle which had the most surface fat.

Different degrees of coagulation of semitendinosus muscle showed that press fluid and total moisture are both decreased with each increment in internal temperature (Table 3) with one exception. No difference was found between meat coagulated to 58°C.(136°F.) and that coagulated to 67°C.(153°F.). Howe (1927) has stated that retention or loss of water by meat is affected by extent of coagulation of protein because this affects ease of evaporation. Total losses, as one would expect, are increased with increased internal temperature (Table 3).

With increased internal temperature the semitendinosus muscle was found to become more tender until 75°C.(167°F.) was reached, when the meat was found to be less tender than that cooked to 67°C. (Table 4). Tenderness is probably a result of several factors. Hydrolysis of collagen increases tenderness, while coagulation of muscle proteins increases density and decreases tenderness. During the first two stages of coagulation, to 58°C. and to 67°C., the effect of hydrolysis is evidently greater than that of increased density from coagulation; while in the last stage, to 75°C., the reverse is true. One, therefore, might expect very tender cuts, containing little collagen, to become toughened by complete coagulation, while less tender cuts may be made more tender. However, much more work should be carried out with different degrees of coagulation and on a number of cuts before conclusive statements can be made about the effect of coagulation on tenderness of meat.

The diameter of muscle fibers is decreased with increased coagulation up to 67°C., but no difference was found between meat coag-

TABLE 3

*Press Fluid, Total Moisture, and Total Losses of Semitendinosus Muscle of Beef,
Raw and Heated to 58, 67, and 75°C.(136, 153, and 167°F.)*

Series no.	Press fluid				Total moisture				Total losses		
	Raw	58°C.	67°C.	75°C.	Raw	58°C.	67°C.	75°C.	58°C.	67°C.	75°C.
	pct. (1)	pct. (2)	pct. (3)	pct. (4)	pct. (1)	pct. (2)	pct. (3)	pct. (4)	pct. (1)	pct. (2)	pct. (3)
1.....	55.11	48.49	49.96	39.95	75.62	71.16	71.16	67.97	19.64	23.52	32.12
2.....	57.21	53.93	53.85	42.93	74.72	71.79	70.93	69.05	14.02	19.96	26.07
3.....	54.12	49.96	51.06	46.37	74.02	70.22	69.45	68.97	19.92	23.72	26.97
4.....	62.87	59.30	53.79	50.94	74.85	73.23	69.67	65.66	16.14	22.85	29.96
5.....	55.69	54.72	54.72	49.60	75.51	72.37	72.62	68.37	17.98	22.20	29.78
6.....	49.82	43.20	50.78	39.34	74.43	69.12	70.42	66.93	18.57	21.45	28.22
7.....	53.74	51.68	49.44	45.23	75.11	71.40	70.79	68.06	16.92	23.09	28.93
8.....	51.35	48.84	53.58	41.55	74.51	69.70	71.50	66.12	18.60	19.46	26.67
9.....	53.76	53.40	50.26	34.40	76.17	71.15	68.25	63.70	19.67	22.91	33.01
10.....	56.31	54.18	53.46	39.88	74.41	71.07	71.96	65.57	16.48	20.36	29.26
11.....	56.78	49.67	48.59	34.33	74.93	70.38	70.04	64.67	18.86	22.73	33.46
12.....	50.85	54.81	50.04	46.93	70.76	69.71	66.58	67.90
Mean.....	54.80 (1)-(2)	51.77 (2)-(3)	51.63 (3)-(4)	42.62	74.59 (1)-(2)	70.94 (2)-(3)	70.28 (3)-(4)	66.91	17.89 (1)-(2)	22.02 (2)-(3)	29.49
d.....	3.03 ¹	0.14	9.01 ¹	3.65 ¹	0.66	3.37 ¹	-4.13 ¹	-7.47 ¹

¹ Very significant difference.

TABLE 4

*Diameter of Muscle Fibers and Tenderness (Shear Force) of Semiindiosus
Muscle of Beef, Raw and Heated to 58, 67, and 75°C.
(136, 153, and 167°F.)*

Series No.	Diameter muscle fibers				Shear force			
	Raw	58°C.	67°C.	75°C. ¹	Raw	58°C.	67°C.	75°C.
	microns (1)	microns (2)	microns (3)	microns (4)	lb. (1)	lb. (2)	lb. (3)	lb. (4)
1.....	62.52	50.20	39.75	42.89	31.90	21.10	18.50	21.40
2.....	60.73	58.78	49.52	52.66	26.00	17.90	14.90	16.20
3.....	65.83	57.33	45.10	50.62	26.00	16.00	17.00	17.80
4.....	56.40	47.22	47.56	44.59	22.50	14.40	15.40	15.40
5.....	57.08	54.28	53.43	44.34	18.60	11.80	10.90	11.20
6.....	73.76	63.56	56.21	59.74	40.34	30.58	26.71	27.63
7.....	61.38	61.77	54.25	56.42	41.54	29.25	23.84	30.46
8.....	65.41	56.35	52.35	53.46	47.69	37.32	31.54	34.25
9.....	71.33	64.13	64.20	65.91	34.67	31.29	27.41	31.71
10.....	65.77	63.41	52.79	53.50	39.04	32.50	22.17	28.42
11.....	67.38	64.13	56.93	59.92	48.95	35.87	30.95	34.09
12.....	67.27	64.70	56.60	58.06	35.04	37.92	22.67	24.75
Mean.....	64.57 (1)-(2)	58.82 (2)-(3)	52.39 (3)-(4)	53.51	34.35 (1)-(2)	26.33 (2)-(3)	21.83 (3)-(4)	24.44
d.....	5.75 ²	6.43 ²	—1.12	8.02 ²	4.50 ²	—2.61 ²

¹ Fibers were not round and probably were measured at widest diameter. ² Very significant difference.

ulated to 67°C. and that coagulated to 75°C. (Table 4). Evidently shrinkage owing to coagulation of muscle plasma is complete at 67°C.

SUMMARY

During the coagulation to 58°C.(136°F.) of longissimus dorsi, triceps brachii, and adductor muscles about four per cent of the total moisture is lost; ether extractable material is increased about 35 to 40 per cent of the original amount; and diameter of muscle cells is decreased from 12 to 16 per cent. The tenderness of the triceps brachii and adductor muscles is not affected, but the longissimus dorsi muscle is made more tender by coagulation to 58°C.

With increasing degrees of coagulation of semitendinosus muscle, i.e., to 58, 67, and 75°C.(136, 153, and 167°F.), press fluid and total moisture are decreased with each increment of internal temperature, except between 58 and 67°C. where no difference was noted; and total losses are increased with each increment in internal temperature. The diameter of muscle fibers is decreased and tenderness is increased with coagulation up to 67°C. The diameter of fiber is not changed between 67 and 75°C. and tenderness is decreased from 67 to 75°C.

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PROBLEMS IN MEAT RESEARCH

I. FOUR COMPARABLE CUTS FROM ONE ANIMAL

II. RELIABILITY OF JUDGES' SCORES¹

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In meat research two questions are repeatedly asked: "Can more than two comparable cuts (lefts and rights) be obtained from one animal?" and "How does subjective judging of meat quality by use of the grading sheet compare with results obtained from objective measurement of these qualities by use of mechanical apparatus?" It is hoped the data in the present study will contribute something to the answers.

EXPERIMENTAL PROCEDURE

In answer to the first question, the longissimus dorsi muscles of pork and of beef were studied to find out whether or not four comparable cuts could be obtained from one animal.

Eleven pairs of center cuts of pork loins, containing four thoracic and four lumbar vertebrae, were ripened for four days at 2 to 3°C. (36 to 37°F.) and cut between the thoracic and lumbar vertebrae, which gave roasts weighing from two to two and one-half pounds each. The roast containing the lumbar vertebrae is hereafter referred to as loin end and the one containing the thoracic vertebrae as rib end.

The 7-8th, 9-10th, and 11-12th standing beef ribs were obtained from the right side of 13 carcasses which graded high-medium to good and which had ripened 12 days at 2 to 3°C.

In answer to the second question, the longissimus dorsi, adductor, and triceps brachii muscles from two classes of beef, namely, cows graded good and steers graded high-medium to good, were used.

Comparableness of Cuts: The meat was cooked in electric Despatch ovens according to methods recommended by the cooking committee of the Cooperative Meat Investigations reported by Alexander, Clark, and Howe (1933), and all cooking records were kept. Pork loins were cooked to 84°C.(183°F.). The two roasts from the left loin of each pair were cooked at 150°C.(302°F.), and those from the right at 200°C.(392°F.), thus making a series of 22 loins since the left and right loins are considered comparable. The meat was cooled to 80°C.

¹ Scientific Journal Series paper No. 1599, Minnesota Agricultural Experiment Station.

² Deceased July 10, 1938.

MEAT COOKING RECORD
GRADING CHART FOR COOKED MEAT¹

Factor	Phase	Very pronounced	Pronounced	Moderately pronounced	Slightly pronounced	Perceptible	Slightly perceptible	Imperceptible
Aroma	Intensity	Very pronounced	Pronounced	Moderately pronounced	Slightly pronounced	Perceptible	Slightly perceptible	Imperceptible
	Desirability	Very desirable	Desirable	Moderately desirable	Slightly desirable	Neutral	Slightly undesirable	Undesirable
Texture	Intensity	Very fine	Fine	Moderately fine	Slightly coarse	Coarse	Very coarse	Extremely coarse
	Intensity	Very pronounced	Pronounced	Moderately pronounced	Slightly desirable	Neutral	Slightly undesirable	Undesirable
Flavor of fat	Desirability	Very desirable	Desirable	Moderately desirable	Slightly desirable	Neutral	Slightly undesirable	Undesirable
	Intensity	Very pronounced	Pronounced	Moderately pronounced	Slightly pronounced	Perceptible	Slightly perceptible	Imperceptible
Flavor of lean	Desirability	Very desirable	Desirable	Moderately desirable	Slightly desirable	Neutral	Slightly undesirable	Undesirable
	Intensity	Very tender	Tender	Moderately tender	Slightly tough	Tough	Very tough	Extremely tough
Quality of juice	Intensity	Very rich	Rich	Moderately rich	Slightly rich	Perceptible	Slightly perceptible	Imperceptible
	Desirability	Very desirable	Desirable	Moderately desirable	Slightly desirable	Neutral	Slightly undesirable	Undesirable
Quantity of juice	Intensity	Very large	Large	Moderately large	Slightly large	Small	Very small	Negligible
	Desirability	Very desirable	Desirable	Moderately desirable	Slightly desirable	Neutral	Slightly undesirable	Undesirable

Place the number of the meat sample above the word which best describes your opinion of the quality.

¹ Grading Sheet of Cooperative Meat Investigations Committee.

(176°F.). Beef ribs were cooked to an internal temperature of 58°C. (136°F.) at 150°C. and cooled to 40°C. (104°F.).

The bone and fat were removed from the cooled roasts and the muscle was halved through the thermometer hole, perpendicular to the muscle fibers. A slice 1.25 cm. thick was cut, from which two samples on either side of the thermometer hole were taken with a borer, 1.25 cm. in diameter, parallel to the muscle fibers for press-fluid determinations. The remainder of the slice, except for one-half inch around the edge, was ground and used for determination of total moisture, ether extract, and total nitrogen. From the other half of the roast two samples for tenderness were taken with a borer, one inch in diameter, parallel to the muscle fibers.

Press Fluid: Each sample was weighed, wrapped in unsized filter cloth, and pressed 10 minutes in the pressometer, developed by Child and Baldelli (1934), under a pressure of 250 pounds. The difference in weight before and after pressing was referred to as press fluid.

Tenderness: Each sample was sheared through the center and through the center of each half by the modification of the Warner-Bratzler shear-stress apparatus which was developed by Child, at the Minnesota Agricultural Experiment Station, and described by Child and Satorius (1938a).

Total Moisture: By Method A two samples, weighing three to five grams each, were dried for 24 hours at 65°C. (149°F.) under a pressure of 25 to 30 millimeters. By Method B two samples, weighing three to five grams each, were dried for two hours in an air oven at 60 to 62°C. (140 to 144°F.) and for five hours at 100°C. (212°F.) under a pressure of 25 to 30 millimeters.

Ether Extract: By Method A two samples of fresh ground meat, weighing two to three grams each, were extracted with ether by the Soxhlet method as described in the official methods of the Association of Official Agricultural Chemists (1930), but checks were so poor that Method B was initiated. By Method B¹ a sample weighing about 10 grams was rubbed with sand in an evaporating dish and dried over night at 60 to 62°C. in an air oven. It was ground with petroleum ether and transferred to a 100-c.c. volumetric flask. This was left over night at a temperature of about 40°C. (104°F.), made to volume at 20°C. (68°F.), and 35-c.c. samples were measured into weighing cans which were dried at 100°C. The amounts extracted by the two methods were compared on 12 samples of meat. The "t" test showed no difference in the average amount extracted although better checks were obtained in Method B.

¹Through the courtesy of the control laboratory of Swift and Company, Chicago, Illinois.

TABLE 1

Press Fluid, Total Moisture, Ether Extract, Total Nitrogen, and Total Losses of Rib and Loin Ends of Center Pork Loin Cooked to an Internal Temperature of 84°C.(183°F.) at 150°C.(302°F.)

Sample No.	Press fluid		Total moisture		Ether extract		Total nitrogen		Total Losses	
	Rib end	Loin end	Rib end	Loin end	Rib end	Loin end	Rib end	Loin end	Rib end	Loin end
1.....	pct. 45.56	pct. 43.07	pct. 61.81	pct. 63.50	pct. 8.68	pct. 4.75	pct. 4.38	pct. 4.79	pct. 18.72	pct. 18.90
2.....	44.94	47.43	6.89	6.55	4.71	4.77	18.54	20.04
3.....	41.89	46.10	60.02	61.73	8.50	5.75	4.91	4.83	21.01	19.80
4.....	46.61	38.39	61.72	60.29	7.43	6.47	4.58	5.01	22.99	26.88
5.....	46.15	47.24	58.41	60.37	8.59	6.70	23.06	24.53
6.....	46.74	36.32	62.32	60.67	5.50	5.31	4.92	5.00	19.82	26.24
7.....	44.71	45.49	62.78	62.02	6.10	7.39	4.81	4.85	17.15	19.68
8.....	44.95	48.75	64.31	67.09	3.10	4.24	4.77	4.75	17.69	17.78
9.....	47.51	50.97	67.86	66.28	5.03	3.65	4.60	4.55	14.47	15.10
10.....	46.59	46.96	63.85	63.57	4.81	5.13	4.64	4.71	18.34	17.60
11.....	51.35	50.18	62.23	59.39	8.69	11.65	4.13	4.55	19.52	22.83
12.....	43.81	40.93	59.78	59.73	12.10	6.71	4.72	5.11	24.70	30.88
13.....	44.09	43.45	59.21	60.88	6.45	7.23	4.63	4.76	25.21	27.43
14.....	40.28	38.99	58.75	60.84	9.35	5.69	4.52	5.07	28.03	25.00
15.....	44.59	41.26	62.07	60.65	6.10	5.25	4.78	5.06	28.78	31.39
16.....	42.61	47.18	56.91	60.52	7.55	7.73	5.06	4.97	30.29	26.56
17.....	47.01	44.52	63.19	62.25	5.87	6.23	4.86	4.63	25.14	25.39
18.....	43.21	39.82	60.15	59.87	8.97	7.03	5.13	5.12	23.91	24.08
19.....	45.49	46.84	64.51	65.69	5.90	3.89	4.77	4.91	20.75	21.92
20.....	48.30	48.83	66.95	64.87	4.93	4.20	4.79	5.02	20.07	25.48
21.....	47.09	51.64	62.66	64.54	6.00	5.18	4.79	4.74	21.55	22.58
22.....	50.61	45.84	60.67	58.33	8.95	12.11	4.40	4.69	25.22	28.85
Mean.....	45.64	45.01	61.91	62.05	7.07	6.31	4.71 ¹	4.85	22.04 ²	23.59

¹ Very significant difference.

² Significant difference.

Total Nitrogen: Total nitrogen was determined on two samples, weighing about one gram each, by the Kjeldahl-Gunning method described in the official methods of the Association of Official Agricultural Chemists (1930).

Press fluid, total moisture (Method A), ether extract (Method A), and total nitrogen were determined for pork; and press fluid, tenderness, total moisture (Method B), and ether extract (Methods A and B) for beef.

Subjective vs. Objective Judging: The meat was prepared, cooked, and sampled as described above for beef ribs except that the meat was judged and sampled at maximum temperature.

Subjective Judging: The grading sheet (Meat Cooking Record Grading Chart for Cooked Meat) recommended by the cooking committee of the Cooperative Meat Investigations, reported by Alexander, Clark, and Howe (1933), was used. Five judges from the Animal Husbandry and Home Economics Departments of the U. S. Department of Agriculture judged the meat, and their scores were averaged for statistical analysis.

Objective Judging: Press fluid, shear stress, and ether extract were determined by the methods described above, Method B being used for ether extract. Diameter and number per bundle of muscle fibers were measured¹ by means of an eye-piece micrometer as described by Brady (1937).

DISCUSSION OF RESULTS

Comparableness of Cuts: Press fluid, total moisture, and ether extract did not differ in the rib and loin ends of center pork loin cooked to 84°C.(183°F.) (Table 1). Total nitrogen and total losses, however, were slightly higher for the loin ends. Total nitrogen is not thought to be an important factor in the physical properties of meat. If the rib and loin ends are rotated in an experiment the small difference in cooking losses need not be considered. Thus the rib and loin ends of the center cuts of pork may be used as comparable roasts in work in which only physical qualities of meat are measured, if the order of roasts is rotated.

Press fluid was higher and cooking losses lower in the 11-12th ribs of beef than in the 9-10th or 7-8th ribs, between which there were no differences. Tenderness in pounds of shear force did not differ among the three cuts. Ether extract was higher in the 7-8th ribs than in the 9-10th or 11-12th ribs, and total moisture was lowest in the 7-8th, higher in the 9-10th, and highest in the 11-12th ribs (Table 2).

¹ Experimental work done by D. E. Brady, Animal Husbandry Department, University of Minnesota.

TABLE 2

Press Fluid, Ether Extract, Total Moisture, Total Losses, and Shear Force of 7-8th, 9-10th, and 11-12th Standing Beef Ribs Heated to an Internal Temperature of 58°C.(136°F.) at 150°C.(302°F.)

Series No.	Press fluid			Total moisture			Ether extract			Shear force			Total losses		
	11-12th	9-10th	7-8th	11-12th	9-10th	7-8th	11-12th	9-10th	7-8th	11-12th	9-10th	7-8th	11-12th	9-10th	7-8th
	pct. (1)	pct. (2)	pct. (3)	pct. (1)	pct. (2)	pct. (3)	pct. (1)	pct. (2)	pct. (3)	lb. (1)	lb. (2)	lb. (3)	pct. (1)	pct. (2)	pct. (3)
1.....	57.17	56.62	53.55	70.46	70.38	69.32	3.39	3.07	2.95	8.43	8.93	10.41
2.....	51.42	46.08	48.09	71.55	70.92	71.31	3.86	3.61	4.61	14.90	17.50	18.70	8.79	11.78	11.17
3.....	54.66	51.06	48.34	69.85	69.59	69.43	3.61	5.40	5.68	14.20	16.70	19.30	9.15	11.60	11.75
4.....	53.98	51.86	50.69	70.98	70.54	71.36	5.34	4.73	3.90	15.50	16.50	14.60	10.59	12.51	12.85
5.....	56.04	50.30	52.57	73.06	72.09	70.07	4.30	3.85	6.99	10.00	8.98	10.13	12.54
6.....	53.14	51.64	48.23	69.15	68.31	67.11	5.82	6.34	7.03	13.00	13.00	16.20	8.69	10.60	13.87
7.....	52.76	48.22	49.89	69.32	67.22	64.78	5.11	8.03	11.59	19.96	22.58	16.04	8.80	11.91	12.42
8.....	51.42	41.76	46.89	69.96	68.04	65.35	4.28	5.89	8.82	22.71	18.09	22.42	11.77	15.41	14.73
9.....	57.02	46.67	50.95	67.47	66.37	64.67	8.95	7.55	9.55	24.55	25.96	24.50	12.32	15.48	14.65
10.....	56.11	50.60	49.93	67.08	67.15	64.53	11.35	7.15	10.93	18.21	16.38	19.42	18.63	17.40	15.17
11.....	55.87	56.51	54.80	70.50	69.84	69.19	6.40	5.94	10.10	17.09	18.55	20.13	10.39	11.55	11.31
12.....	56.71	49.08	50.15	68.87	68.52	66.93	7.17	6.42	10.47	21.71	22.63	21.59	13.41	17.65	15.84
13.....	55.91	53.41	49.00	66.99	61.30	59.24	11.81	16.23	17.33	23.33	19.25	19.33	14.07	18.50	17.26
Mean.....	54.79	50.29	50.24	69.63	68.48	67.18	6.26	6.48	8.46	17.93	18.83	19.29	11.08	13.34	13.38
t.....	4.82 ¹	0.07	9.23 ¹	2.75 ²	4.15 ¹	4.03 ¹	0.37	4.14 ¹	3.25 ¹	0.23	0.49	0.71	5.11 ¹	0.09	4.15 ¹
	(1)-(2)	(2)-(3)	(1)-(3)	(1)-(2)	(2)-(3)	(1)-(3)	(1)-(2)	(2)-(3)	(1)-(3)	(1)-(2)	(2)-(3)	(1)-(3)	(1)-(2)	(2)-(3)	(1)-(3)

¹ Very significant difference. ² Significant difference.

Thus for work involving only physical properties, i.e., press fluid, tenderness, and cooking losses, the 7-8th and 9-10th ribs can be used as comparable; but if chemical analyses are involved, none of the three cuts are comparable. The cuts should always be rotated, however.

Subjective vs. Objective Judging: Meat quality, as studied by Child and Satorius (1938b), of two classes of animals judged by the grading sheet and by objective methods brought forth interesting results when studied by analysis of variance. Although no difference was found between the two classes in press fluid, judges' scores for juiciness showed Class II, beef from cows graded good, to be less juicy than Class I, beef from steers graded high-medium to good. Consequently, no correlation was found between press fluid and palatability-juiciness (Table 3). Evidently juiciness as judged must be affected by other factors. A highly positive correlation was found between palatability-juiciness and the flavor-aroma of the meat. It was suggested by Coffey and Augustus (1917) that juiciness and flavor are closely associated. It is interesting to note that no correlation existed between press fluid and flavor-aroma of meat. Palatability-juiciness (quantity) and quality of juice also showed a remarkably high correlation, about $+0.8$. Juiciness does not seem to be closely associated with ether extract (fat content), however, as has been suggested by Howe (1927, 1937), for no correlation was found between press fluid and ether extract or between quality of juice and ether extract (Table 3). Even a negative correlation was found between quantity of juice (grading sheet) and ether extract. Thus, from these correlations we may say that palatability-juiciness is greatly affected by other factors of palatability.

Palatability-tenderness showed a high correlation with pounds of shear force (Table 3). The correlation is negative because the higher the tenderness score the lower the pounds of shear force. Tenderness and texture as measured by the grading sheet are highly correlated, $+0.56$. It is interesting to note that both texture and tenderness (shear stress) show significant correlations with diameter of muscle fiber and number of fibers per bundle. Thus, the smaller the fiber and the more fibers per bundle the more tender will be the meat and the finer will be the texture.

Although flavor and aroma are judged separately on the grading sheet, it is well conceded that the two senses—taste and smell—are hard to separate. A high correlation, $+0.7$, was found between these factors so the two factors were combined for further correlations. Flavor of meat was thought by Howe (1937) to be related to lubrication of lipids, to tenderness, and to texture. No correlation was found

between flavor-aroma and texture. Flavor-aroma and tenderness are positively correlated, and flavor-aroma and ether extract are negatively correlated. Thus, meat of better flavor in this case was judged to be more tender but contained less fat.

It has been suggested by Hall¹ (1937) that tenderness and juiciness may be associated. A significant positive correlation was found

TABLE 3

Coefficients of Correlation Among Factors of Palatability of Meat as Measured by Grading Sheet and by Mechanical Methods

Correlation between	Number of samples	r	P is less than
Press fluid and quantity of juice (score card).....	39	.31	n.s. ¹
Flavor-aroma and quality-quantity of juice.....	39	.68	.01
Flavor-aroma and press fluid.....	39	.01	n.s.
Quality of juice and quantity of juice (score card)..	39	.79	.01
Ether extract and press fluid.....	39	.27	n.s.
Ether extract and quantity of juice (score card).....	39	— .35	.05
Ether extract and quality of juice.....	39	— .23	n.s.
Shear stress and tenderness (score card).....	36	— .69	.01
Texture and tenderness (score card).....	39	.56	.01
Shear stress and diameter of muscle fibers.....	48	.53	.01
Shear stress and number per bundle of muscle fibers	48	— .64	.01
Texture and diameter of muscle fiber.....	39	— .43	.01
Texture and number per bundle of muscle fibers.....	39	.76	.01
Flavor and aroma.....	39	.70	.01
Flavor-aroma and texture.....	39	.18	n.s.
Flavor-aroma and tenderness (score card).....	39	.52	.01
Flavor-aroma and ether extract.....	39	— .47	.01
Shear stress and press fluid.....	36	— .21	n.s.
Tenderness (score card) and quantity of juice (score card).....	39	.51	.01

¹ n.s. = non-significant.

between these two factors as judged by the score card. However, no correlation was found when mechanical methods were used, i.e., no correlation was obtained between press fluid and mechanical shear.

CONCLUSIONS

The longissimus dorsi muscles of pork and beef have been found homogenous in physical properties so that four comparable roasts may be obtained from one animal of either pork or beef. The rib and loin ends, weighing about two pounds each, were found comparable from the center pork loin; and the 7-8th and 9-10th ribs, weighing seven and six pounds respectively, were found comparable from beef. The order of roasts should always be rotated in the experiment.

¹ Personal communication.

Factors of palatability as measured by the grading sheet are interrelated. Such an interdependence was found between flavor and aroma, quantity and quality of juice, flavor-aroma and juiciness (quality and quantity), texture and tenderness, flavor-aroma and tenderness, and tenderness and juiciness. In the case where mechanical methods were involved, no such relationship existed, i.e., between press fluid and shear stress, and between press fluid and flavor-aroma.

The grading sheet is an accurate measure of tenderness, since a high correlation exists between grading sheet scores and mechanical shear. No correlation is found, however, between quantity of juice as judged by grading sheet scores and press fluid. It is realized, also, that "juiciness" as judged may justly involve other palatability factors, such as flavor-aroma, which stimulate the flow of saliva; while press fluid can mean only the amount of juice expressed under the given conditions.

Other factors, such as flavor-aroma, are more important in affecting juiciness than ether extract.

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PREPARATION OF ICE CREAM MIXES FOR HOME CONSUMPTION

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Even though vast improvements have been made in the manufacture and merchandising of ice cream, there is still a large number of housewives who prefer to make their own frozen desserts. In a survey conducted by Longstaff (1935) of the University of Minnesota, it was found that approximately 20 per cent of the families questioned made their own ice cream. Similar findings were reported by Smith (1937) of the University of Illinois. In the Illinois study it was found that 50 per cent of those making their own ice cream did so because they thought the homemade product was of better quality than the commercial product. Other common reasons given were greater confidence in their own make, cheapness, and greater variety of flavors. One of the main reasons given for not using more commercial ice cream was the inconvenience of purchasing. Sixty-four per cent of all those questioned indicated that they would be interested in having a prepared ice cream mix delivered to them on the milk route. It would seem from these studies that a prepared ice cream mix suitable for home consumption would fill a definite need in the dairy industry.

Various attempts have been made to supply prepared or desiccated mixes for household use, but commercially none of these products have been entirely successful. Conover (1935) demonstrated that a mix could be sterilized in small hermetically sealed cans. He found that the cooked flavor could be minimized by using a high temperature, short-time sterilization. He also observed that the cooked taste was further minimized when such flavors as chocolate, lemon, orange, pistachio, caramel, raspberry, or maple were added to the mix before sterilization. The sterilization procedure found most suitable was to heat the mix rapidly to 87.8°C.(190°F.), then heat to 115.6°C.(240°F.) at the rate of 2.8°C.(5°F.) per minute and hold at this temperature for four minutes, then raise the temperature to 126.7°C.(260°F.) in three minutes, and cool immediately to room temperature.

Some of the problems involved in the manufacture of sterilized, canned mix are heat coagulation, cooked flavor, progressive fat ris-

ing, and destabilization of protein. In order to obtain more definite information concerning the above problems and their solution this study was undertaken.

EXPERIMENTAL PROCEDURE

The mixes were prepared in small batches from fresh 40-per cent cream, skim milk, and plain condensed or sweetened condensed skim. Batches were pasteurized at 71.1°C.(160°F.) for 30 minutes, homogenized at the above temperature, using 2,000 pounds of pressure on the first valve and 500 pounds on the second valve. The mixes were cooled and canned in pint (No. 2) tin cans or in quart (No. 3) cans. The cans were sealed with a hand sealer. The mixes were sterilized in a small pilot Fort Wayne Sterilizer which has a capacity of 32 (No. 2) cans.

The standard sterilization procedure which was used, unless otherwise stated, was as follows:

1. The sterilizer was filled half full of water and then steam was turned on and the temperature raised to 87.8°C.(190°F.) as rapidly as possible.
2. The temperature was raised 2.8°C.(5°F.) per minute from 87.8°C.(190°F.) to 115.6°C.(240°F.).
3. The temperature was held at 115.6°C.(240°F.) for four minutes.
4. After holding at 115.6°C.(240°F.) for four minutes the temperature was raised to 126.7°C.(260°F.) at the rate of 3.9°C.(7°F.) per minute.
5. The temperature was held at 126.7°C.(260°F.) for one minute and was then lowered to room temperature as rapidly as possible.

TEMPERATURE OF STERILIZATION

Sterilization of a product by heat is a time and temperature process. If the temperature is increased the time can be correspondingly decreased and vice versa. In the selection of a sterilization temperature for ice cream mix it is necessary to consider the effect of the heat on the flavor and body of the mix, and at the same time the heat treatment must be sufficient to produce a sterile product.

Generally speaking, high temperature, short-time sterilization produces less cooked flavor than a lower temperature for a longer period. It is for this reason that a modified, short-time, high-temperature heat treatment was attempted for the sterilization of canned mix. Using Conover's procedure of sterilization [that is, heating rapidly to 87.8°C.(190°F.), then raising the temperature to 115.6°C.(240°F.) at the rate of 2.8°C.(5°F.) per minute, holding at 112.6°C.(240°F.)

for four minutes, increasing the temperature to 126.7°C. (260°F.) in three minutes' time, then cooling immediately after reaching 126.7°C. (260°F.)] did not result in complete sterilization in every case. It was found, however, that if after reaching 126.7°C. (260°F.) the sterilizer was held at that temperature for one minute before cooling, no spoilage occurred in any of the tests. This procedure has a minimum effect on flavor and was adopted as the most promising for the sterilization of ice cream mix.

RELATION OF COMPOSITION TO HEAT COAGULATION

Heat coagulation is a problem in the manufacture of evaporated milk, but this can be overcome by proper regulation of acidity, salt

TABLE 1

Effect of Mix Composition on Heat Coagulation of Ice Cream Mix

Mix No.	Fat	Serum solids	Sugar	Total solids	After sterilization	
					Flavor	Body
	<i>pct.</i>	<i>pct.</i>	<i>pct.</i>	<i>pct.</i>		
1.....	12	7	15	34	Satisfactory	Satisfactory
2.....	12	9	15	36	Satisfactory	Satisfactory
3.....	12	11	15	38	Satisfactory	Sl. curdy
4.....	12	9	12	33	Satisfactory	Satisfactory
5.....	12	9	14	35	Satisfactory	Satisfactory
6.....	12	9	18	39	Satisfactory	Sl. curdy
7.....	16	7	15	38	Satisfactory	Curdy
8.....	16	9	15	40	Satisfactory	Curdy
9.....	16	11	15	42	Satisfactory	Curdy
10.....	16	9	12	37	Satisfactory	Curdy
11.....	16	9	0	25	Satisfactory	Satisfactory
12.....	16	9	10	35	Satisfactory	Satisfactory
13.....	16	9	14	39	Satisfactory	Sl. curdy
14.....	16	9	18	43	Satisfactory	Curdy
15.....	20	9	0	29	Satisfactory	Satisfactory

balance, and sterilization temperature. In the case of ice cream mix the problem is slightly greater because of a higher fat and total solids content. In order to determine the effect of each mix constituent on the heat-coagulation point, a number of mixes of varying composition were made from 40-per cent cream, concentrated skim milk, skim milk, and sugar. They were processed and sterilized in the regular manner.

The data show that as the total solids are increased the danger of heat coagulation is also increased (Table 1). The serum solids have, without doubt, the greatest effect on the heat stability. The fat is the next most important factor and the sugar, the least important.

From the data secured in this study and from that of Conover, it is evident that it is practically impossible to sterilize successfully mixes containing over approximately 36 per cent of total solids. Mixes containing near the upper limit in total solids should not contain over 10 per cent of the serum solids to avoid a low heat-coagulation point.

RELATION OF HOMOGENIZATION PRESSURE TO
HEAT COAGULATION

That homogenization destabilizes the protein of many dairy products has been shown conclusively. By means of the alcohol test Doan (1929) has been able to measure the destabilization of milk caused

TABLE 2

Effect of Homogenizing Pressure on Heat-Coagulation Point of Ice Cream Mix
(Mix contained 12% fat, 9% serum solids, and 15% sugar)

Mix No.	Total pressure on first valve	Pressure on second valve	Appearance after sterilizing
	<i>lb.</i>	<i>lb.</i>	
1.....	Satisfactory
2.....	2,000	Curdled
3.....	3,000	Curdled
4.....	4,000	Curdled
5.....	2,000	500	Satisfactory
6.....	2,500	500	Satisfactory
7.....	2,000	1,000	Curdled
8.....	3,000	1,000	Curdled

by homogenization. Tracy and Ruehe (1930), in studying the effect of different factors on cream feathering, found high homogenization pressures detrimental to the stability of coffee cream.

As it seemed likely that high homogenizing pressures would lower the heat-coagulation point of ice cream mixes which are to be sterilized, a study was made to determine the effect of different homogenizing pressures on heat coagulation. The mix was prepared from 40-per cent cream, concentrated skim, skim milk, and sugar. The composition was 12 per cent fat, 9 per cent serum solids, and 15 per cent sugar. The mix was pasteurized and homogenized at 71.1°C. (160°F.). Before homogenization the mix was divided into eight portions, and different homogenizing pressures were used on each portion. After processing, the mixes were canned in No. 2 tin cans and sterilized in the Fort Wayne sterilizer, using the standard procedure.

The data show conclusively that homogenization has an appreciable effect on the heat-coagulation point of ice cream mix (Table 2). The unhomogenized mix sterilized with no coagulation, but an un-

homogenized sterilized mix, would not be desirable because of the cream plug that would form and the churning that would take place. The use of pressure on a single valve caused coagulation, the degree of coagulation being greater at the higher homogenizing pressures. Higher pressures on the second valve also caused coagulation during sterilization. A combination of 2,000 or 2,500 pounds on the first valve and 500 pounds on the second valve gave the best results.

EFFECT OF STABILIZERS ON HEAT COAGULATION

Stabilizers of one kind or another are used almost universally in commercial ice cream for the purpose of absorbing some of the free

TABLE 3

Effect of Stabilizers on Heat-Coagulation Point of Ice Cream Mix

Mix No.	Fat	Serum solids	Sugar	Stabilizer	Degree of coagulation
	pct.	pct.	pct.	pct.	
1.....	12	9	13.5	None	None
2.....	12	9	13.5	.15 Dariloid	Sl. heavy body
3.....	12	9	13.5	.20 Dariloid	Sl. heavy body
4.....	12	9	13.5	.30 Dariloid	Sl. curdled
5.....	12	9	13.5	.40 Dariloid	Sl. curdled
6.....	12	9	15.0	None	None
7.....	12	9	15.0	.3 gelatin	None
8.....	12	9	15.0	.6 gelatin	Cheesy, curdled
9.....	12	9	15.0	.2 Dariloid	Spongy, curdled
10.....	12	9	15.0	.4 Dariloid	Spongy, curdled
11.....	12	9	15.0	.2 Cocoloid	Spongy, curdled
12.....	12	9	15.0	.4 Cocoloid	Spongy, curdled

water and improving the body of the ice cream. So far as we know, the effect mix stabilizers might have on the heat-coagulation point of milk products has never been studied. In order to determine the effect of added stabilizers on protein stability, various amounts of gelatin, Dariloid,¹ and Cocoloid¹ were used. The composition of the mixes and the amount of stabilizer used are given (Table 3). The mixes were made from 40-per cent cream, concentrated skim, skim milk, and sugar. They were pasteurized at 71.1°C.(160°F.) and homogenized at 2,000 and 500 pounds of pressure on the first and second valves respectively.

That the stabilizers lower the heat-coagulation point is evident from the data (Table 3). All three of the stabilizers used exhibited approximately the same amount of destabilization, although the types of bodies of the mixes were somewhat different.

¹ Sodium alginate product.

EFFECT OF EGG YOLK ON HEAT COAGULATION

Egg yolk is widely used in commercial ice cream because of the beneficial effect it has on the whipping qualities of the mix. Since it was found that the other mix constituents had an important effect on the heat stability of the mix, it was thought desirable to determine the effect of added egg yolk. To a mix containing 12 per cent fat, 9 per cent serum solids, and 15 per cent sugar various amounts of dried egg yolk were added. The mix was prepared from 40-per cent cream, concentrated skim, skim milk, and sugar. It was pasteurized, homogenized, and sterilized in the usual manner. The data show that egg yolk also lowers the heat-coagulation point of ice cream mixes (Table 4).

TABLE 4

Effect of Egg Yolk on Heat-Coagulation Point of Ice Cream Mix

Mix No.	Fat	Serum solids	Sugar	Egg yolk	Degree of coagulation
	<i>pct.</i>	<i>pct.</i>	<i>pct.</i>	<i>pct.</i>	
1.....	12	9	15	None
2.....	12	9	15	.40 powdered	Sl. curdled
3.....	12	9	15	.75 powdered	Curdled
4.....	12	9	15	1.00 fresh	Curdled

EFFECT OF STORAGE TEMPERATURE ON CERTAIN PHYSICAL AND CHEMICAL PROPERTIES OF STERILIZED MIX

In order to study the storage qualities of sterilized mix, one containing 12 per cent fat, 9 per cent serum solids, and 15 per cent sugar was divided into two lots, one of which was stored at 4.4°C. (40°F.) and the other at room temperature. At the end of a four-months period it was observed that the mix stored at 40°F. was in every respect the same as it was when fresh, while that kept at the higher temperature had acquired a storage flavor, was slightly darker in color, and had a distinct cream plug. The pH and titratable acidity did not change appreciably during storage at either temperature.

In order to obtain more complete information relative to the physical changes that take place during storage a mix containing 10.25 per cent fat, 9 per cent serum solids, and 14 per cent sugar was divided into two lots, one of which was stored at 4.4°C. (40°F.) and the other at room temperature or approximately 26.7°C. (80°F.). Two cans of mix at each storage temperature were opened each week and flavor, body, and color were noted; and pH, titratable acidity, and degree of fat rising were determined. To test for fat rising, the bottom of an undisturbed can was punctured with an ice pick and a

vent made in the top of the can. Successive 200-c.c. portions were drained from the can and tested for fat content by the Mojonnier method. The results for the first ten observations are given (Table 5).

It will be noted that the most change occurred in the samples stored at 26.7°C.(80°F.). In these samples the color darkened slightly, a storage taste developed, and there was a decided fat separation. From these data it would seem that the original flavor and appearance can be better preserved by storing at the lower temperature.

FLAVORING THE MIX

Adding various flavors to the mix before sterilization helps to mask the cooked flavor resulting from the high heat treatment. Certain precautions should be taken, however, in the selection of flavors so as not to lower the heat-coagulation point or injure the flavor. If alcoholic solutions of vanilla flavor are used, an objectionable medicinal flavor is produced during sterilization. A water solution of vanillin can be satisfactorily used.

Fruits or nuts cannot be added to the mix before sterilization as they lower the heat-coagulation point of the mix to such an extent that coagulation takes place.

In this study it was found that mixes which had been flavored with caramel, maple, raspberry, chocolate, lemon, orange, and pistachio could be successfully sterilized. A commercially prepared caramel flavor was successfully used, but excessive amounts of burnt sugar caused coagulation. A true concentrate was used for the maple flavor. For raspberry a natural-flavor concentrate syrup gave good results. Use of chocolate liquor lowered the heat-coagulation point. A good chocolate mix was made by increasing the sugar content of the mix 1.25 per cent and using 1.25 per cent of high-quality cocoa. A lemon extract and yellow coloring were used to flavor and color the lemon mix and an orange extract and orange coloring were used to flavor and color the orange mix. For pistachio, an imitation pistachio flavor was used and the mix was tinted with green color.

There are undoubtedly other flavors that can be used successfully in a sterilized mix, but the above are especially recommended.

MARKETING POSSIBILITIES

In order to test the sales possibilities of this product, No. 2 cans of plain mix, chocolate, raspberry, orange, lemon, pistachio, caramel, and maple were placed on the market at a retail price of 16 cents per can. A mix containing 12 per cent fat, 9 per cent serum solids, and 14.5 per cent sugar was sold through the University of Illinois agricultural products salesroom and on the University retail milk

TABLE 5
Effect of Storage Temperature on Acidity, Color, Flavor, Appearance, and Fat Rising of Sterilized Mix

Week	Titratable acidity		pH		Color		Flavor		Appearance		Fat test (Original test, 10.25%)					
											80°F.			40°F.		
	80°F.	40°F.	80°F.	40°F.	80°F.	40°F.	80°F.	40°F.	80°F.	40°F.	1st 200 c.c.	2nd 200 c.c.	3rd 200 c.c.	1st 200 c.c.	2nd 200 c.c.	3rd 200 c.c.
	pct.	pct.									pct.	pct.	pct.	pct.	pct.	pct.
1st	.18	.18	6.18	6.18	1	1	1	1	1	1	9.65	10.56	10.95	9.98	10.25	10.05
2nd	.19	.19	6.20	6.24	1	1	1	1	1	1	8.95	10.33	10.94	9.81	10.29	10.43
3rd	.20	.19	6.227	6.26	Sl. darker	1	Sl. off	1	1	1	8.97	10.19	11.43	9.50	10.17	11.70
4th	.20	.18	6.26	6.27	Sl. darker	1	Sl. off	1	1	1	8.47	9.94	11.95	9.28	10.00	11.08
5th	.19	.19	6.24	6.24	Sl. darker	1	Sl. off	1	Sl. fat plug	1	7.60	9.34	14.76	9.25	9.99	11.33
6th	.19	.18	6.37	6.32	Sl. darker	1	Sl. off	1	Fat plug	1	6.37	8.97	17.54	9.19	9.89	11.80
7th	.19	.19	6.30	6.32	Sl. darker	1	Sl. off	1	Fat plug	1	5.69	8.18	18.13	9.10	9.85	12.29
8th	.18	.18	6.29	6.39	Sl. darker	1	Sl. off	1	Fat plug	1	5.29	7.90	17.21	8.56	9.74	12.08
9th	.18	.18	6.29	6.32	Sl. darker	1	Sl. off	1	Fat plug	1	4.38	7.24	18.28	7.98	9.88	14.93
12th	.18	.18	6.32	6.41	Sl. darker	1	Sl. off	1	Fat plug	1	4.42	7.29	19.24	6.90	9.04	14.27

¹ Satisfactory.

route. In the month of July, approximately 700 cans were marketed through these channels. (This was the only month in which it was sold.) Though some adverse criticisms were received, there were many repeat purchases. It would seem that a product which sold so readily through these channels without any special sales effort being made would have considerable sales possibilities.

SUMMARY AND CONCLUSIONS

It has been shown that an ice cream mix can be successfully processed and sterilized; however, it is advisable to

1. Limit the total solids to 36 per cent and the serum solids to 9 per cent. (A mix containing 12 per cent fat, 9 per cent serum solids, and 14.5 per cent sugar was found to give the most satisfactory results.)

2. Use fresh, high-quality dairy products.

3. Homogenize at 2,000 to 2,500 pounds of pressure on the first valve and 500 pounds on the second valve.

4. Use the short-time, high-temperature sterilization method.

There are certain flavors that are more suitable for use than others. Mixes properly flavored and sterilized will have only a slightly cooked flavor.

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THERMAL PROCESSING OF CANNED FOODS IN TIN CONTAINERS

I. VARIATION OF HEATING RATE WITH CAN SIZE FOR PRODUCTS HEATING BY CONVECTION

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In calculating a thermal process for canned foods according to the methods given by Ball (1923, 1928), it is necessary to know the temperature of the food in the can during sterilization. The temperature, as a function of the time, is found experimentally by heat-penetration tests in which a sealed can of the food under consideration is given a process similar to that which it will receive commercially. The temperature of a point in the can (usually the slowest heating point) may be read at any instant by means of a thermocouple whose hot junction is inside the can, the indicating instrument being some distance away. Sometimes these tests are made with only one size of can, and at a later date it may be desirable to know what the temperature as a function of the time would be if the same product were packed in a can of different size. It is therefore desirable to have some means of converting heat-penetration data obtained for one can size to the equivalent for another can size.

A method has been devised for obtaining the desired information without the necessity of repeating the test with the new can size, Ball (1923), but this procedure gives reliable results only when the product is of heavy consistency so that the transfer of heat takes place by conduction. It is the purpose of this paper to explain a method which seems to give results more nearly in accord with experimental data when the heating of the food takes place by convection.

If we let RT be the retort temperature and CT be the can temperature, and then plot $RT-CT$ against the time t on semi-logarithmic paper with properly chosen scales, the resulting graph is essentially a straight line or, in some instances, two intersecting straight lines. The number of minutes required for the straight line to traverse one common logarithmic cycle is designated by f_h and is often referred to as the slope of the line. For conduction heating, the variation of f_h with the can size has been studied and experimentally confirmed by Ball (1923).

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During conduction heating, transfer of heat into the can takes place without actual transfer of material and during most or all of the sterilization process there is a difference in temperature between the center of the can and a point near the outside. The center temperature increases only as long as this difference exists, since heat flows only from a place of high temperature to one of a lower temperature. On the other hand, when heating is by convection, the rise in temperature at the center of the can is due principally to the actual transfer of heated material from the hotter portions of the can. Most liquid foods heat by a combination of conduction and convection. If the food is of heavy consistency, conduction is the most important factor and large temperature differences are present within the can. If the food is fairly thin, convection plays a more important part and the temperature differences within the can are not so great. That thin products actually do heat by convection is shown by the fact that their heating is so rapid as to be unaccounted for by thermal conductivity alone. Furthermore, tests have shown that in the heating of thin liquids in cans, temperature differences between any two points in the can are much smaller than in the heating of solids or heavy liquids. Typical products heating by conduction are cream-style corn, pumpkin, and dog food; products heating principally by convection are broths, brine-packed peas, and beer.

As a limiting case of convection heating (likewise mechanical agitation), the temperature in the can will at any instant be everywhere the same. It must be understood that this is a situation which is only approximated in practice and which might be considered to represent ideal thermal convection.

DERIVATION OF EQUATIONS

Assuming the conditions of ideal thermal convection, let T represent the temperature of the contents of the can at time t . When $t = 0$, we assume that the surface of the can is instantly raised to temperature RT and is maintained at this temperature thereafter. Let H be the quantity of heat in the contents of the can. This is directly proportional to the volume and the temperature of the can contents, or

$$H = AvT + \text{constant} \quad (1)$$

where A is a constant for a given product and v is the volume of the can. The rate at which heat enters a unit area of the surface of the can will be proportional to the temperature difference $RT - T$. Then the rate at which the quantity of heat H increases is

$$\frac{dH}{dt} = BS(RT - T), \quad (2)$$

where S is the surface area of the can and B is a factor of proportionality. Differentiating (1) and substituting in (2) we obtain

$$\frac{dT}{dt} = \frac{BS}{Av} (RT - T). \quad (3)$$

The integral of (3) is

$$T = RT - (RT - IT) e^{-\frac{BS}{Av} t},$$

or

$$T = RT - (RT - IT) \cdot 10^{-\frac{BS}{MAv} t}. \quad (4)$$

The constant of integration is chosen so that $T = IT$ when $t = 0$. In the second form of (4), $M = \log_e 10$.

Referring to (4) and the definition of f_h , we find that

$$f_h = \frac{MAv}{BS}. \quad (5)$$

Suppose now that two cans of different sizes are processed in such a manner that RT and IT are the same for both. If we let f_h , S , v , and f_h' , S' , v' be the quantities corresponding to the two cans, we have from (5)

$$f_h = \frac{MAv}{BS}, \quad (6)$$

$$f_h' = \frac{MAv'}{BS'}.$$

Since A and B are the same for both cans

$$\frac{f_h}{f_h'} = \frac{vS'}{Sv'}. \quad (7)$$

If the cans are cylindrical, we can express the surfaces and volumes in terms of the radius r and the length l . Then (7) becomes

$$\frac{f_h}{f_h'} = \frac{rl}{r + l} \cdot \frac{r' + l'}{r'l'}. \quad (8)$$

The quantity $\frac{rl}{r + l}$ which we refer to as the *can factor*, may be com-

puted from a knowledge of the dimensions of the can. When these values are substituted in (8), the numerical value of the right mem-

ber is known. Then if f_h' is known from experiment, f_h may be computed from (8).

APPLICATION

A list of computed values of $\frac{rl}{r+1}$ for various can sizes is given

(Table 1). As an example of the practical application of this table, the solution of a typical problem is given below.

By experiment, it is found that for a certain product in a 401 x 411 size can, $f_h = 2.9$. What is the corresponding f_h in a 603 x 700 can?

TABLE 1
Can Factors for Convection Heating

Can size	Factor	Can size	Factor
202 x 214	0.7292	307 x 306	1.0603
202 x 308	0.7744	307 x 400	1.1292
208 x 211	0.7868	307 x 408	1.1735
211 x 300	0.8629	307 x 409	1.1785
211 x 304	0.8883	307 x 510	1.2496
211 x 400	0.9490	307 x 512	1.2566
211 x 414	0.9999	307 x 604	1.2821
211 x 600	1.0464	401 x 411	1.3412
300 x 407	1.0593	404 x 414	1.4169
300 x 409	1.0676	502 x 510	1.7356
303 x 406	1.1020	603 x 408	1.7410
303 x 509	1.1761	603 x 700	2.0708
303 x 512	1.1855	603 x 812	2.2156
307 x 302	1.0276		

The above can sizes are expressed in the notation commonly used in the canning industry. The first number represents the over-all diameter and the second number represents the over-all length. In each number, the first digit denotes whole inches while the second and third denote sixteenths of an inch. Thus, a 202 x 214 can is $2\frac{1}{16}$ inches in diameter and $21\frac{1}{16}$ inches high.

We find the can factors 1.3412 and 2.0708 for the 401 x 411 and 607 x 700 cans, respectively (Table 1). These values substituted in (8) give

$$f_h = f_h' \frac{rl}{r+1} \cdot \frac{r'+1}{r'l'} = 2.9 \frac{2.0708}{1.3412} = 4.5.$$

Experimentally, it was found that $f_h = 4.7$. If can factors for conduction heating are used, $f_h = 6.9$, by calculation.

SUMMARY

A method is given for converting heat-penetration data obtained for one can size to the equivalent for another can size when the can

contents heat mainly by convection. This method gives results which agree well with experimental data.

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CHEMICAL AND BACTERIOLOGICAL STUDIES ON ICE CREAM

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An investigation of ice cream on the commercial market was carried out in the spring of 1937 from the consumer's standpoint, since it was felt that previous studies had seldom been aimed directly toward the satisfaction of the consumer. In this study the percentages of various constituents in a number of leading commercial ice creams on the market in metropolitan Chicago were experimentally determined with the object of relating the results to subjective standards of quality. The analyses in this study were confined to vanilla ice cream, since it constitutes from 50 to 60 per cent of the ice cream produced and may therefore be considered representative.

CHEMICAL ANALYSES OF COMMERCIAL ICE CREAM

Since sweetness is a factor in taste appeal, the ice creams were analyzed for sugar content. For this determination the polariscope method, advocated by the Association of Official Agricultural Chemists, was used. The averaged results obtained show that although there is a great variation among the ice creams, three groups emerge so far as the percentage of this ingredient is concerned (Table 1). Brands A, B, and C rank at the top with the highest content, D and E in the middle, and the remaining ice creams—F, G, H, and I—at the bottom of the list. Those ice creams with low sugar (sucrose) content may, however, have cerelese (corn sugar) present, in which case the total percentage would be increased. So far as sweetness was ranked in the subjective tests, there seemed to be little agreement, an ice cream with a low sugar content being marked "sweet" by a judge who did not seem to think that an ice cream with a higher percentage of sugar was too sweet. It is believed that this result may be explained by the proportion of other ingredients in the ice creams. It is also possible that some of the judges lacked a keen sense of taste.

The percentage of butterfat in the ice creams was determined also, since the average person considers the quantity of this ingredient present to be a leading criterion of quality. The method used for the determination was the Minnesota test, a modification of the

TABLE 1
Summary of Experimental Work on Ice Creams Purchased in Metropolitan Chicago

Brand	How purchased	Sugar (sucrose)	Fat	Fat value ¹	Total solids	Serum solids	Overrun	Yield per penny
		pct.	pct.	gm.	pct.	pct.	pct.	gm.
A.....	Packaged pint	16.8	14.2	1.96	39.0	8.0	91.5	13.8
B.....	Packaged pint	16.6	14.4	1.87	39.5	8.5	87.5	13.0
C.....	Packaged pint	16.1	13.7	1.77	39.2	9.4	87.5	12.9
D.....	Bulk pint	14.2	25.3	2.96	40.0	0.5	69.8	11.7
E.....	Packaged pint	14.0	13.0	2.25	35.8	8.8	100.0	17.3
F.....	Bulk pint	11.7	12.6	1.78	40.8	16.5 ²	16.8	14.1
G.....	Packaged pint	11.1	16.3	2.02	38.2	10.8	87.5	12.4
H.....	Packaged pint	10.8	12.8	1.73	38.4	14.8 ²	143.0	13.5
I.....	Bulk pint	10.8	12.8	1.93	38.4	14.8 ²	14.1	15.1
	Packaged pint	10.4	12.9	1.66	37.8	14.5 ²	100.0	12.9

¹The value received, in terms of fat, for each penny spent on the ice cream. ²These products obviously include cereose, inasmuch as the amount of sugar is low and the amount of serum solids as reported is considerably higher than that ordinarily used in ice cream.

Babcock. The averaged results (Table 1) show that there is over 100 per cent variation among the ice creams tested, Brand D being at the top with 25.3 per cent and Brand F at the bottom with 12.6 per cent. The average range is 12.6 to 14.4 per cent, however. By calculation with relation to price per pint, fat value was obtained; figuring on this basis Brand I was lowest and Brand D highest (Table 1). The interesting point brought out here is that one ice cream with a smaller percentage of fat than another may actually give the consumer more fat value for his money. It is of further interest to note that all the ice creams comply with both the Illinois and the federal standards of 12 per cent.

Experimentation also covered total solids, because of the manufacturers' desire to increase this amount to the limit, for the following reasons: sugar, quite often used in so doing, is inexpensive; the flavor is richer; and the body and texture are improved materially. A modification of the Association of Official Agricultural Chemists' method, as commonly used by the ice cream industry, was employed for the determination of this important factor. The averaged results (Table 1) may be seen to exceed even the highest minimum standard set by a state legislature. Also, Brand E is found to be lowest with 35.8 per cent, while Brand F is highest with 40.8 per cent. The average range is 37.8 to 39.5 per cent. Brand A, with 39 per cent, was scored highest in the subjective tests on the basis of its body and texture. Hence, since body and texture are known to be improved by a high solids content, within certain limits, the percentage of solids contained in Brand A is obviously that for which the manufacturer should strive, since it produced an ice cream having the most appeal to consumers.

Total solids were also determined for the reason that from them an approximation might be obtained of the amount of ingredients other than fat and sugar which were present. These other ingredients are commonly spoken of as milk-solids-not-fat or serum solids. (In the results obtained for this factor may be included small amounts of reducing sugar and stabilizer.) These serum solids contain the food values which build bones and teeth, build and repair tissue, and serve as fuel. Hence, those ice creams which rank high in milk-solids-not-fat—namely, Brands G, I, H, packaged and bulk, and F—are most suitable for the myriads of students and office workers who make their lunch almost wholly on ice cream. The other ice creams still retain their very legitimate position as palatable desserts, however, and after all, their appeal to the palate is the chief reason for their purchase by consumers. Finally, it is of interest to note in connection with these serum solids that they

show quite obviously what Brand D really is—a frozen, sweetened cream.

WIDE VARIETY IN BACTERIAL COUNTS

Bacterial counts were taken on all the ice creams, first, because bacteria are likely to cause flavor defects and, second, because ice cream is a potential carrier of disease. The method used was the standard "Plate Colony Count" as outlined by the American Public Health Association. With the exception of those for Brand D, the results (Table 2) are well under even the lowest state maximum tolerated, which is 100,000 per gram. This standard is also in effect in Chicago. Brand D, however, exceeds even the highest maximum count, which is 500,000 per gram. Brand A, it is interesting to note,

TABLE 2

Bacterial Counts of Ice Creams Purchased in Metropolitan Chicago

Brand	How purchased	Range in count per gram	Average count per gram
A.....	Packaged pint	1,200- 3,200	1,900
B.....	Packaged pint	5,200- 48,000	21,000
C.....	Packaged pint	5,000	5,000
D.....	Bulk quart	74,000- 366,000	173,000
	Bulk pint	17,500-7,000,000	1,913,000
E.....	Packaged pint	6,000- 9,600	7,800
F.....	Packaged pint	11,000- 14,000	12,000
	Bulk pint	6,400- 175,000	51,600
G.....	Packaged pint	3,200- 3,500	3,300
H.....	Packaged pint	2,600- 110,000	74,800
	Bulk pint	2,000- 15,000	7,900
I.....	Packaged pint	10,000- 60,000	27,400
L.....	Dixie cup	6,200- 8,500	7,400

came consistently lowest in count with an average of 1,900 bacteria per gram, a figure which places it in Sommer's classification as almost a rare case. Using the subjective tests as criteria, there seems to be justification, on the whole, for assuming that the number of bacteria present did not influence the flavor of the ice creams examined.

Another fact brought out in this study, which is of special interest to the consumer, is the direct correlation existing between the number of bacteria found and the manner in which the ice cream is sold. In other words, packaged pints were, on the whole, low in bacterial count, while the bulk pints were generally high in count. The magnitude of the count in bulk ice cream also varies within a sample, probably for the following reasons: (1) the length of time during which the first scoop taken out has been on top of the batch; (2) the time the sample touches the dipper, whether just after the

latter is taken from its container of usually murky water or after several scoops have been made; and (3) the conjunction of the sample with the dipper, i.e., its location in the scoopful. Variation in counts between samples of a packaged ice cream was also found and is attributed to season, weather conditions, cleanliness of the store, and length of time following delivery. The conclusion drawn is that some regulation of the bacterial count is advisable; such legislation is believed necessary to spur retailers on to more careful handling of bulk ice cream, which has been shown to be the chief offender as regards high bacterial count.

WEIGHT DIFFERENCES IN PACKAGED SAMPLES

The weights of the packages of ice cream (Table 3) reveal further facts of interest to the consumer. They show a considerable varia-

TABLE 3
Weights of Pints of Ice Cream Purchased in Metropolitan Chicago

Brand	How purchased	Samples weighed	Range in weight
			<i>gm.</i>
A.....	Packaged	7	248.8-310.7
B.....	Packaged	5	274.2-304.6
C.....	Packaged	5	272.8-307.6
D.....	Bulk	4	317.5-393.3
E.....	Packaged	3	260.6-290.2
F.....	Packaged	1	266.6
	Bulk	3	437.1-480.2
G.....	Packaged	2	272.5-274.3
H.....	Packaged	2	227.4-242.9
	Bulk	3	390.2-463.4
I.....	Packaged	4	257.9-301.0
J.....	Packaged	1	208.5
K.....	Packaged	1	291.3
	Bulk	1	465.0

tion in the quantity of ice cream, as measured by weight, which the purchaser receives for his money when he buys ice cream by volume, i.e., in pint or quart measures. Between two pints of different brands, as great a difference as 200 grams was found. Furthermore, such a variation was not limited to different brands, two pints of the same brand—ready-packaged, not bulk—varying in weight from each other by 35 grams. These facts indicate that it is time for the consumer to begin a campaign for the selling of ice cream by weight rather than volume; the manufacturers would have to listen and the consumer would be well rewarded.

Variation was also found in the amount of ice cream which the consumer obtains for each penny he spends (Table 1). In other words, putting aside the question of quality, if the consumer is mainly interested in getting the most ice cream for his money, he can get as much as 5.6 grams more of ice cream by buying Brand E rather than Brand D, the ice creams with the greatest difference in yields per penny.

Since incorporation of air in the freezing operation is important because of its direct relation to yield and profit and its effect on body, texture, and palatability, the amount of air whipped into the ice cream was determined. This amount is expressed as per cent overrun. Only Brand J and one Brand H sample exceeded the per cent overrun tolerated by the federal government and those states with legal requirements. The unusually low percentages obtained on the bulk ice creams H and F are quite significant. They may be explained on the basis of difference in price charged and method of handling the bulk as compared with the packaged ice cream. The former, as is obvious from the weights of the pints, are packed down into the container and may, in addition, have undergone shrinkage while in the retailer's cabinet. A further explanation is that the ice cream melted and was then refrozen. Another point of interest in this connection is that the results of the subjective tests showed that ice cream with an overrun between 76 and 92 per cent is most likely to meet favor with the consumer.

SUMMARY

Thirty-nine per cent seems to be the amount of total solids and between 76 and 92 per cent the amount of overrun which the consumer most favors. Sugar remains a matter of individual preference, while much the same may be said about fat, although the latter is not liked in the extremely high quantity in which it is found in Brand D. Taken as a whole, the proportion of ingredients which produces the best ice cream, as judged by the results of the subjective tests, is that which constitutes Brands F, B, A, E, I, C, and G, in practically this same order.

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INDEX TO VOLUME 3

Abrams, Allen. Packaging materials and their application to the food industry	239
Acid-base balance of cereals.....	393
Action of enzymes at low temperatures.....	57
Action of guaiacum.....	555
Adhesion of potato-tuber cells.....	513
Air conditioning in food plants.....	127
Alcohol production by <i>Saccharomyces ellipsoideus</i>	453
Alcoholic liquors, aldehyde in.....	499
Amerine, M. A., and Winkler, A. J. Color in California wines. I. Methods for measurement of color.....	429
——, and ——, Color in California wines. II. Preliminary comparison of certain factors influencing color.....	439
Ammonia in decomposed haddock.....	589
Antirachitic effect of some foods.....	373
Apparatus for tenderness.....	221
Apple juice, preservation.....	467
Arnold, Aaron, and Elvehjem, C. A. Stability of vitamin B ₁ of vacuum-dried animal tissues during storage.....	367
Art of buttermaking.....	261
Ascorbic acid in frozen foods.....	109
Ascorbic acid in frozen peas.....	489
Assay of foods for iron.....	383
Available iron in foods.....	383
Ayers, S. Henry. Recent developments in canning fruit juices.....	5
Bacteria in frozen vegetables.....	299
Bactericidal activity of crotonaldehyde.....	389
Bacteriological examination of ice cream.....	653
Bacteriology of processing.....	21
Ball, C. O. Advancement in sterilization methods for canned foods.....	13
Balls, A. K., and Lineweaver, Hans. Action of enzymes at low temperatures....	57
Barnby, H. A., Food preservation in modern glass containers.....	240
Baselt, F. C., Townsend, C. T., and Esty, J. R. Heat-resistance studies on spores of putrefactive anaerobes in relation to determination of safe processes for canned foods.....	323
Beavens, E. Arthur, Goresline, Harry E., and Pederson, Carl S. Preservation of grape juice. V. Pasteurization of grape and apple juices for storage or immediate fermentation.....	467
Beef muscle.....	619
Beef, sampling.....	505
Bergstrom, Paul, Johnson, Victor, Carlson, Anton J., and Kleitman, Nathaniel. Action of gum guaiacum upon the animal organism.....	555
Berube, Louis. Modern practice in fish preservation by cold.....	69
Better meals for tomorrow.....	276
Bioassay of foods for iron.....	383
Bitting, A. W. Present trends in canning.....	242
Boiling, effect on vitamin C in carrots.....	403
Bonar, Lee, and Mrak, E. M. A note on yeast obtained from slimy sausage....	615
Bottling industry, scientific control.....	141

Brewing technology.....	269
Brooks, J. Color of meat.....	75
Burton, L. V. Importance of the unit operation concept in food engineering....	79
Buttermaking, art of.....	261
Cabbage, vitamin C in cooked.....	311
California wines, color in.....	429, 439
Cameron, E. J. Recent developments in canning technology with reference to spoilage control.....	91
Camp, S. C., King, C. G., Fenton, Faith, and Tressler, D. K. Losses of vitamin C during boiling and steaming of carrots.....	403
Can size and heating rate.....	647
Can manufacture.....	253
Canned foods, processing.....	647
——, safe processes.....	323
Canned food sterilization.....	13
Canning cheddar cheese.....	267
Canning fruit juices.....	5
Canning, metallurgy in.....	278
Canning of fish.....	273
Canning technology in spoilage control.....	91
Canning, trends in.....	242
Carlson, Anton J., Kleitman, Nathaniel, Bergstrom, Paul, and Johnson, Victor. Action of gum guaiacum upon the animal organism.....	555
Carotene content of frozen foods.....	109
Carrots, effect of cooking on vitamins in.....	403
Cereals, acid-base balance.....	393
Cheddar cheese canning.....	267
Cheese canning.....	267
Chemical examination of ice cream.....	653
Chewing resistance of foodstuffs.....	221
Child, Alice M., and Moyer, Elsie Zohner. Variations in sampling beef and pork roasts for press-fluid investigations.....	505
——, and Satorius, Mary J. Effect of coagulation on press fluid, shear force, muscle-cell diameter, and composition of beef muscle.....	619
and ———. Problems in meat research. I. Four comparable cuts from one animal. II. Reliability of judges' scores.....	627
Chlorine in honey.....	543
Citrus fruits, reducing substances.....	351
Cold, effect on proteins.....	161
Cold, fish preservation.....	69
Cold injury of fruits.....	175
Cold storage of herring.....	205
Color in California wines.....	429
Color of meat.....	75
Colorimetric investigation of foods.....	155
Commercial freezing, effect on vitamin C.....	409
Conference, food technology.....	1
Convection	647
Cooking, effect on vitamin C in carrots.....	403
——, effect on vitamin C in peas.....	409
——, effect on vitamin C in vegetables.....	311

Corbett, W. J., and Tracy, P. H. Preparation of ice cream mixes for home consumption	637
Creamery butter, microbiological examination of.....	287
Crooks, G. Chapman, and Ritchie, W. S. A study of rate of decomposition of haddock muscle at various temperatures as indicated by ammonia content	589
Crotonaldehyde, bactericidal activity.....	389
Crystallization of proteins.....	161
Dack, G. M., and Davison, Ellen. Salmonella food poisoning—infection or intoxication?	347
Dairy industry problems	259
Dairy industry technology.....	245
Dairy technology.....	211
Davidson, Jehiel, and LeClerc, J. A. Acid-base balance of cereals and some related food materials.....	393
Davison, Ellen, and Dack, G. M. Salmonella food poisoning—Infection or intoxication?	347
Decomposition of haddock.....	589
Defrosting, effect on vitamin C in peas.....	409
Dessication of proteins.....	161
Detection of thermophilic bacteria in skim-milk powder.....	421
Development of pink color in sauerkraut.....	583
DeWitt, James B., and Sure, Barnett. A study of certain reducing substances in citrus fruits.....	351
Diastatic activity of honey.....	539
Dietz, Carl. Iodine content of some Ohio vegetables.....	359
Disposal of food-plant wastes.....	227
Domestic refrigeration of foods.....	199
Dung, halophilic bacteria in.....	417
Eddy, W. H., White, Mary E., Sanborn, N. H., and Kohman, E. F. The anti-rachitic effect of some foods.....	373
Effect of cooking vegetables on vitamin C in.....	311
Eggs, gas storage.....	149
Eggs, shell, storage of.....	599
Elvehjem, C. A., and Arnold, Aaron. Stability of vitamin B ₁ of vacuum-dried animal tissues during storage.....	367
Engineering of pasteurization.....	257
Enzymes, action at low temperatures.....	57
Esty, J. R., Baselt, F. C., and Townsend, C. T. Heat-resistance studies on spores of putrefactive anaerobes in relation to determination of safe processes for canned foods.....	323
Evaporated milk	283
Evaporated milk, vitamin D in.....	283
Ewell, Arthur W. Present use and future prospects of ozone in food storage....	101
Examination, microbial, butter.....	287
Fay, A. C. Current technological problems in the dairy and ice cream industries	245
Fellers, C. R., and Fitzgerald, G. A. Carotene and ascorbic acid content of fresh market and commercially frozen fruits and vegetables.....	109
Fenton, Faith, and Tressler, D. K. Losses of vitamin C during commercial freezing, defrosting, and cooking of frosted peas.....	409
—, —, Camp S. C., and King, C. G. Losses of vitamin C during boiling and steaming of carrots.....	403

Fessler, J. F., and Mrak, E. M. Changes in iron content of musts and wines during vinification.....	307
Fermentation, lactic acid bacteria in.....	317
Fiber container for fluid milk.....	248
Fiber milk container.....	248
Fincke, M. L., and Garrison, E. A. Utilization of calcium of spinach and kale	575
Fish canning.....	273
Fish preservation by cold.....	69
Fishery products, proteins.....	549
Fitzgerald, F. F. Development of a fiber container for fluid milk.....	248
Fitzgerald, G. A., and Fellers, C. R. Carotene and ascorbic acid content of fresh market and commercially frozen fruits and vegetables.....	109
——, Jenkins, R. R., and Tressler, D. K. Vitamin C content of vegetables.	
VIII. Frozen peas	133
Food engineering, unit operation.....	79
Food-plant wastes	227
Food plants, air conditioning.....	127
Food poisoning, salmonella.....	347
Food preservation, glass containers.....	240
——, microbiology	189
Food research, spectroscopy in.....	121
Food spoilage, lactic acid bacteria in.....	317
Food storage and ozone.....	101
Food technology conference.....	1
Foods, antirachitic effect.....	373
——, calorimetric investigation.....	155
——, iron in.....	383
——, refrigeration domestic.....	199
Foodstuffs, tenderness.....	221
Foter, Milton J., and Golick, Ann M. Inhibitory properties of horse-radish vapors	609
Freezing, effect on vitamin C in peas.....	409
Freezing of herring.....	205
Frozen foods, carotene content.....	109
Frozen peas, vitamin C content of.....	133, 409
——, vitamins in.....	489
Frozen vegetables, bacteria in.....	299
Fruit, cold injury.....	175
Fruit juices, canning.....	5
Fruits, frozen, ascorbic acid in.....	109
——, frozen, carotene content of.....	109
Garrison, E. A., and Fincke, M. L. Utilization of calcium of spinach and kale	575
Gas storage, meat and eggs.....	149
Glass containers.....	240
Golick, Ann M., and Foter, Milton J. Inhibitory properties of horse-radish vapors	609
Goresline, Harry E., Pederson, Carl S., and Beavens, E. Arthur. Preservation of grape juice. V. Pasteurization of grape and apple juices for storage or immediate fermentation.....	487
Grape juice, pasteurization.....	487

Greenlie, David G., and Proctor, Bernard E. Determination of optimum conditions for domestic refrigeration of foods.....	199
Grumbine, Cecily Ruth, and Halliday, Evelyn G. Chemical and bacteriological studies on ice cream.....	653
Guaiacum, action of.....	555
Haddock muscle, rate of decomposition.....	589
Halliday, Evelyn G., and Grumbine, Cecily Ruth. Chemical and bacteriological studies on ice cream.....	653
Halophilic bacteria, isolation of.....	417
Harris, Philip L., and Poland, George L. A note on the bioassay technique for determining available iron in foods.....	383
Harrison, George R. Spectroscopy in food research.....	121
Harrison, William H. Research problems of the can manufacturer.....	253
Heat penetration.....	19
Heat penetration in tin cans.....	647
Heat permeability of potato.....	525
Heat resistance studies on spores.....	323
Herring, freezing of.....	205
High-short sterilization.....	28
Hohl, Leonora. Further observations on production of alcohol by <i>Saccharomyces ellipsoideus</i> in syrupe fermentations.....	453
Holmquist, C. A., and Tiedeman, W. D. Engineering of pasteurization.....	257
Holt, James. Air-conditioning for food plants.....	127
Honey, chlorine and sulfur in.....	543
———, pigmentation	539
Horse-radish vapors.....	609
Ice cream.....	653
Ice cream mixes.....	637
Infection in salmonella food illness.....	347
Ingersoll, R. L., Vollrath, R. E., Scott, Bernard, and Lindegren, C. C. Bactericidal activity of crotonaldehyde.....	389
Inhibitory properties of horse-radish vapors.....	609
Intoxication in Salmonella food poisoning.....	347
Iodine content of vegetables.....	359
Iron content of musts.....	307
Iron in foods, assay of.....	383
Irradiated evaporated milk.....	283
Isolation of halophilic bacteria.....	417
Jenkins, R. R., Tressler, D. K., and Fitzgerald, G. A. Vitamin C content of vegetables. VIII. Frozen peas.....	133
Jones, A. H., and Lochhead, A. G. Types of bacteria surviving in frozen-pack vegetables.....	299
Johnson, Victor, Carlson, Anton J., Kleitman, Nathaniel, and Bergstrom, Paul. Action of gum guaiacum upon the animal organism.....	555
Judkins, H. F. Problems yet to be solved in the dairy industry.....	259
Kale, utilization of calcium of.....	575
Kelly, C. D., and Pederson, Carl S. Development of pink color in sauerkraut..	583
Kertesz, Z. I. Pectic enzymes. II. Pectic enzymes of tomatoes.....	481
King, C. G., Fenton, Faith, Tressler, D. K., and Camp, S. C. Losses of vitamin C during boiling and steaming of carrots.....	403
Kleitman, Nathaniel, Bergstrom, Paul, Johnson, Victor, and Carlson, Anton J. Action of gum guaiacum upon the animal organism.....	555

Kohman, E. F., Eddy, W. H., White, Mary E., and Sanborn, N. H. The anti-rachitic effect of some foods.....	373
Krieger, C. H., and Scott, H. T. Stability of vitamin D in irradiated evaporated milk.....	283
Lactic acid bacteria in food spoilage.....	317
Lanham, William B., Jr., and Lemon, James M. Nutritive value for growth of some proteins of fishery products.....	549
Lead in maple syrup.....	449
LeClerc, J. A., and Davidson, Jehiel. Acid-base balance of cereals and some related food materials.....	393
Lemon, James M., and Lanham, William B., Jr. Nutritive value for growth of some proteins of fishery products.....	549
Levine, Max. Application of scientific control in the bottling industry.....	141
Lindegren, C. C., Ingersoll, R. L. Vollrath, R. E., and Scott, Bernard. Bactericidal activity of crotonaldehyde.....	389
Lineweaver, Hans, and Balls, A. K. Action of enzymes at low temperatures....	57
Liquors, aldehydes in.....	499
Lochhead, A. G., and Jones, A. H. Types of bacteria surviving in frozen-pack vegetables	299
Losses of vitamin C during cooking.....	403
Losses of vitamin C in frosted peas.....	409
Low temperatures, enzyme action.....	57
Maple syrup, lead in.....	449
Massachusetts Institute of Technology conference.....	1
Measurement of color in wines.....	429, 439
Meat, color of.....	75
——, gas storage.....	149
——, quick freezing.....	167
Meat research	627
Metallurgy and canning.....	278
Microbiological examination of creamery butter.....	287
Microbiology of food preservation.....	189
Milk container, paper.....	248
Milk-whey in foods.....	233
Mixes, ice cream.....	637
Molds in creamery butter.....	287
Moran, T. Gas storage of meat and eggs.....	149
Moyer, Elsie Zohner, and Child, Alice M. Variations in sampling beef and pork roasts for press-fluid investigations.....	505
Mrak, E. M., and Bonar, Lee. A note on yeast obtained from slimy sausage....	615
——, and Fessler, J. F. Changes in iron content of musts and wines during vinification	307
Muscle, beef	619
Musts, iron in.....	307
Note on yeast from sausage.....	615
Nutritive value of fish proteins.....	549
Olson, F. C. W., and Schultz, O. T. Thermal processing of canned foods in tin containers. I. Variation of heating rate with can size for products heating by convection.....	647
Ozone in food storage.....	101
Packing materials in food industry.....	289
Paper milk container.....	248

Paper wrappings	239
Parker, M. E. Technological exploration of the art of buttermaking.....	261
Pasteurization, engineering	257
Pasteurization of grape juice.....	467
Pork, sampling	505
Pearlstein, Janet F., and Schuette, H. A. Degree of pigmentation and its probable relationship to the diastatic activity of honey.....	539
Peas, frozen, vitamins in.....	489
Pectic enzymes of tomatoes.....	481
Pederson, Carl S. Lactic acid-producing bacteria in fermentations and food spoilage	317
——, Beavens, E. Arthur, and Goresline, Harry E. Preservation of grape juice. V. Pasteurization of grape and apple juices for storage or im- mediate fermentation	467
——, and Kelly, C. D. Development of pink color in sauerkraut.....	583
Perlick, A. Calorimetric investigation of foodstuffs.....	155
Personius, Catherine J., and Sharp, Paul F. Adhesion of potato-tuber cells as influenced by temperature.....	513
——, and ———. Permeability of potato-tuber tissue as influenced by heat	525
Pigmentation of honey.....	539
Piettre, Maurice. Cristallisation et dessiccation de certain proteides sous l'action du froid.....	161
——. Essais de "quick freezing" applique aux grosses pieces de viande.....	167
Pink color of sauerkraut.....	583
Plank, R. Contribution to the theory of cold injury to fruit.....	175
Poland, George L., and Harris, Philip L. A note on the bioassay technique for determining available iron in foods.....	363
Potato-tuber cells, adhesion.....	513
Prescott, Samuel C. Purpose of the food technology conference.....	1
——, and Tanner, Fred W. Microbiology in relation to food preservation...	189
Present trends in canning.....	242
Preservation of fish by cold.....	69
Preservation of grape juice.....	467
Problems in meat research.....	627
Processes, safe	323
Processing canned foods.....	17, 647
Proctor, Bernard E., and Greenlie, David G. Determination of optimum con- ditions for domestic refrigeration of foods.....	199
Proteins, crystallization and dessiccation.....	161
Quick freezing of meats.....	167
Reay, George A. Freezing and cold storage of herring.....	205
Reducing substances in citrus fruits.....	351
Refrigeration of foods.....	199
Research in can manufacture.....	253
Ritchie, W. S., and Crooks, G. Chapman. A study of rate of decomposition of haddock muscle at various temperatures as indicated by ammonia content	589
Rogers, L. A. Canning of cheddar cheese.....	267
<i>Saccharomyces ellipsoideus</i> , alcohol production by.....	453
Safe processes for canned foods.....	323
Salmonella food poisoning.....	347
Sampling beef and pork.....	505

Sanborn, N. H., Kohman, E. F., Eddy, W. H., and White, Mary E. The antirachitic effect of some foods.....	373
Satorius, Mary J., and Child, Alice M. Effect of coagulation on press fluid, shear force, muscle-cell diameter, and composition of beef muscle.....	619
——, and ———. Problems in meat research. I. Four comparable cuts from one animal. II. Reliability of judges' scores.....	627
Sauerkraut, pink color.....	583
Sausage, slimy, yeast from.....	615
Schuette, H. A., and Pearlstein, Janet F. Degree of pigmentation and its probable relationship to the diastatic activity of honey.....	539
——, and Triller, Ralph E. Mineral constituents of honey. III. Sulphur and chlorine	543
Schultz, O. T., and Olson, F. C. W. Thermal processing of canned foods in tin containers. I. Variation of heating rate with can size for products heating by convection.....	647
Scientific control in bottling industry.....	141
Scores of meat.....	627
Scott, Bernard, Lindegren, C. C., Ingersoll, R. L., and Vollrath, R. E. Bactericidal activity of crotonaldehyde.....	389
Scott, H. T., and Krieger, C. H. Stability of vitamin D in irradiated evaporated milk	283
Shadwick, G. W., Jr. A study of comparative methods and media used in microbiological examination of creamery butter.....	287
Sharp, Paul F., and Personius, Catherine J. Adhesion of potato-tuber cells as influenced by temperature.....	513
——, and ———. Permeability of potato-tuber tissue as influenced by heat	525
Shell eggs, storage	599
Sippel, George B. Recent advances in brewing technology.....	269
Skim-milk powder, thermophilic bacteria in.....	421
Slimy sausage, yeast from.....	615
Soil, halophilic bacteria in.....	417
Sorenson, C. M. Detecting thermophilic contamination in skim-milk powder... ..	421
Sparling, B. L., and Todhunter, E. N. Vitamin values of garden-type peas preserved by frozen-pack method. I. Ascorbic acid (vitamin C).....	489
Spectroscopy in food research.....	121
Spinach, calcium utilization of.....	575
Spoilage control and canning technology.....	91
Spores, heat resistance.....	323
Stability of vitamin B ₁	367
Stability of vitamin D in evaporated milk.....	283
Steaming, effect on vitamin C in carrots.....	403
Sterilization of canned foods.....	13
Stero-vac process	6
Storage of shell eggs.....	599
Stuart, L. S. Isolation of halophilic bacteria from soil, water, and dung.....	417
Sulfur in honey.....	543
Sure, Barnett, and DeWitt, James B. A study of certain reducing substances in citrus fruits.....	351
Swenson, T. L. Storage of shell eggs.....	599
Tanner, Fred W., and Prescott, Samuel C. Microbiology in relation to food preservation	189

Technology in ice cream industry.....	245
Technology of brewing.....	269
Technology of buttermaking.....	261
Tenderness of foodstuffs.....	221
Theory of cold injury of fruit.....	175
Thermal processing of canned foods.....	647
Thermophilic bacteria in skim-milk powder.....	421
Tin can manufacture.....	253
Tin cans, processing.....	647
Tobey, James A. Some recent advances in dairy technology.....	211
Tobie, Walter C. Improved procedures in determination of aldehydes in distilled alcoholic liquors with Schiff's reagent.....	499
Todhunter, E. N., and Sparling, B. L. Vitamin values of garden-type peas preserved by frozen-pack method. I. Ascorbic acid (vitamin C).....	489
Tomatoes, pectic enzymes in.....	481
Townsend, C. T., Esty, J. R., and Baselt, F. C. Heat-resistance studies on spores of putrefactive anaerobes in relation to determination of safe processes for canned foods.....	323
Tracy, P. H., and Corbett, W. J. Preparation of ice cream mixes for home consumption	637
Trends in canning	242
Tressler, C. J., Jr., and Willits, C. O. Sources of lead in maple syrup and a method for its removal.....	449
Tressler, D. K., Camp, S. C., King, C. G., and Fenton, Faith. Losses of vitamin C during boiling and steaming of carrots.....	403
——, and Fenton, Faith. Losses of vitamin C during commercial freezing, defrosting, and cooking of frosted peas.....	409
——, Fitzgerald, G. A., and Jenkins, R. R. Vitamin C content of vegetables. VIII. Frozen peas.....	133
Tressler, Donald K., and Wellington, Marylizabeth. Vitamin C content of vegetables. IX. Influence of method of cooking on vitamin C content of cabbage	311
Triller, Ralph E., and Schuette, H. A. Mineral constituents of honey. III. Sulphur and chlorine.....	543
Types of bacteria in frozen vegetables.....	299
Unit operation in food engineering.....	79
Utilization of calcium of spinach	575
Utilization of whey in foods.....	233
Vacuum-dried animal tissues.....	367
Variations in meat samples.....	505
Vegetables, frozen, ascorbic acid in.....	109
——, frozen, carotene content of.....	109
——, iodine in.....	359
——, frozen, vitamin C in.....	133
Vitamin B ₁ , stability.....	367
Vitamin C in frozen peas.....	133
Vitamin C in vegetables.....	311
Vitamin C losses in cooking carrots.....	403
Vitamin C losses in frosted peas.....	409
Vitamin D in evaporated milk.....	283
Vitamins in frozen peas.....	489

Vollrath, R. E., Scott, Bernard, Lindegren, C. C., and Ingersoll, R. L. Bactericidal activity of crotonaldehyde.....	389
Volodkevich, N. N. Apparatus for measurements of chewing resistance or tenderness of foodstuffs.....	221
Vucassovich, M. P. Special problems in fish canning.....	273
Warrick, L. F. Disposal of food-plant wastes.....	227
Water, halophilic bacteria in.....	417
Waters, Lewis W. Better meals for tomorrow.....	276
Webb, B. H. Utilization of whey in foods.....	233
Wellington, Marylizabeth, and Tressler, Donald K. Vitamin C content of vegetables. IX. Influence of method of cooking on vitamin C content of cabbage	311
Whey in foods.....	233
White, Mary E., Sanborn, N. H., Kohman, E. F., and Eddy, W. H. The antirachitic effect of some foods.....	373
Williams, Robert S. Research in metallurgy and its significance in canning.....	278
Willits, C. O., and Tressler, C. J., Jr. Sources of lead in maple syrup and a method for its removal.....	449
Wine, iron in.....	307
———, color in.....	429, 439
Winkler, A. J., and Amerine, M. A. Color in California wines. I. Methods for measurement of color.....	429
———, and ———. Color in California wines. II. Preliminary comparisons of certain factors influencing color.....	439
Wrapping, paper.....	239
Yeast from slimy sausage.....	615
Yeasts in creamery butter.....	287

